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MOSAIC *VS.* EQUIPOTENTIAL DEVELOPMENT[1]

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It is interesting to note how old questions and controversies crop up again and again in slightly new form in the progress of science. One of these ageless questions concerns the essential nature of development. In the eighteenth century it was "evolutio" or preformation *vs.* epigenesis. In the late nineteenth and early twentieth centuries it was mosaic *vs.* equipotential development. To-day it is self-differentiation *vs.* embryonic induction.

We now know that these two are not antagonistic or mutually exclusive, but that each is true in part, and the problems of embryonic determination are chiefly those of the time, place and manner in which each is operative. After the absurdities of extreme preformation came the absurdities of extreme epigenesis. Up to the last decade of the nineteenth century orthodox biology held to a rigid belief in extreme epigenesis, and if it did not generally go as far as did Alex. Goette (1875) in claiming that the egg of the toad is unorganized and non-living material until after fertilization, it held firmly to the faith that embryonic differentiation first appeared in the late cleavage or blastula stages and was entirely correlative. The dictum of Driesch was generally accepted by embryologists, namely, that "the fate of a part is a

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function of its position." It is true that there were always representatives of the opposing view of self-differentiation, but in the main they were as voices crying in the wilderness. It was generally held that morphogenesis began with the germ layers of the gastrula. Driesch claimed that the cleavage of the egg was a mere "sundering of homogeneous materials capable of any fate," and W. K. Brooks, at the time (1891) when I presented my doctoral thesis to him, held that "cleavage is a mere vegetative duplication of parts without morphological significance." On the other hand, Wilhelm His (1874) argued on purely logical grounds that there are "Organbildende Keimbezierke" in the blastoderm of the hen's egg and W. Roux (1884), as a result of his notable experiments on the frog's egg, held that from the four-cell stage on the development was a "mosaic work of four independently developing pieces."

Largely under the influence of Professor Whitman, the work of the Woods Hole school of embryologists proved that embryonic differentiation appears in the early cleavage stages and that the early blastomeres of many different species are not totipotent; that even in some unsegmented eggs there are regions and substances that are already differentiated and destined under typical conditions to give rise to specific parts or organs. But on the other hand there were certain groups of animals, particularly coelenterates, echinoderms, nemertines, amphioxus, teleosts and amphibians in which no such early differentiations were shown, and consequently I proposed in 1897 that the former type should be known as "determinate," the latter as "indeterminate." Indeed, I once suggested rather facetiously that "every egg is a law unto itself," and this fly was embalmed in the amber of E. B. Wilson's book on "The Cell." For a time there seemed to be a lack of any general principles as to the time and manner in which embryonic differentiations appear, and one eminent physiologist informed me that

the study of cell-lineage had been a blind alley which led nowhere.

More recent work has shown that many of these so-called indeterminate types of cleavage are not really such, or at least that the cleavage cells are not composed of undifferentiated cytoplasm. For example, in one of the most indeterminate types, the scyphomedusan Linerges, I found (1906) in the unsegmented egg a nearly concentric arrangement of three cytoplasmic substances, namely (1) a peripheral hyaline layer, thickest at the animal pole, which later forms the outer ciliated portion of the ectoderm cells, (2) within this a yolk-rich layer, thickest at the vegetative pole, which later forms the inner ends of the ectoderm cells and most of the endoderm cells, and (3) a central gelatinous area, which later becomes the blastocoel jelly and which by absorbing water causes the enlargement of the blastocoel. It is evident that with such a concentric arrangement of egg substances, large fragments from any portion of the egg would contain portions of all these substances.

The echinoderm egg, upon which more experimental work has been done than upon any other, was held until recently to be a striking example of indeterminate cleavage. Driesch (1891, 1893) at one time maintained that cleavage cells were all alike, "like balls in a pile," but the later works of Runnström (1914), Hörstadius (1928) and Plough (1927) show that as early as the 4- and 8-cell stages the blastomeres of various echinids show marked differentiations. On the other hand, Tennent, Taylor and Whitaker (1929) have proved that before fertilization the egg of Lytechinus may be cut in two in any plane and the fragments remain capable of fertilization and complete development. Still more remarkable is the fact that at the time of operation the polar differentiation of this egg is not fixed, but in each half the chief axis is established perpendicular to the plane of section. Evidently in the interval between the stage at which they

operated and that with which Hörstadius worked a notable degree of differentiation has arisen.

The cytoplasmic substances of many ovarian eggs have a more or less concentric arrangement, which undergoes polar concentration and differentiation at the time of maturation or fertilization. Thus in the eggs of many gasteropods little if any cytoplasmic differentiation is visible until the germinal vesicle dissolves and its clear, hyaline contents spread out over the animal pole and extend about two thirds of the way to the vegetative pole; this hyaline substance goes in large part into the three quartettes of ectomeres, while the yolk-rich substance of the vegetative hemisphere gives rise to the endoderm (Conklin, 1910). The primary mesoderm comes from the left posterior macromere, which bears the polar lobe, and this lobe is visible at each division from the first maturation until the second cleavage; if it is removed mesoderm fails to develop (Crampton, 1896). Therefore, before the first cleavage, substances destined to the formation of all three germinal layers are already present and localized in the gasteropod egg.

In the ascidian egg, which is one of the most highly differentiated types known, the different cytoplasmic substances before maturation are nearly concentric in position; there is a granular peripheral layer which later goes into the mesoderm and may, therefore, be called "mesoplasm,' a hyaline layer thickest at the animal pole, especially after the escape of the contents of the germinal vesicle, which may be called "ectoplasm," since it gives rise chiefly to ectoderm, and an inner yolk-rich layer the "endoplasm," which largely goes into the endoderm. After fertilization and before the first cleavage these concentric layers rapidly take the characteristic positions which they occupy in the embryo and larva. The polar differentiation is accentuated by the flow of mesoplasm to the vegetative pole, then the mesoplasm forms a crescent around the posterior side of the egg, parallel with the first cleavage spindle, and the chorda-neuroplasm

forms a crescent around the anterior side; the endoplasm lies on the vegetative side of these crescents and the ectoplasm on the animal-pole side. Thus bilaterality and the definitive pattern of localization are established.

Destruction of individual cleavage cells of the ascidian egg, while others survive, demonstrates that these cells are specifically differentiated as early as the 2-cell stage, —a right or left 1/2 blastomere develops only a right or left half-larva, anterior or posterior 2/4 blastomeres develop into anterior or posterior half-larvae, etc. Relatively weak centrifuging of ascidian eggs proves that yolk, pigment and mitochondria may be displaced without preventing normal development, but if the localized areas of hyaloplasmic substances are displaced by strong centrifugal force, corresponding organs of the larva are displaced. In a recent masterly work Dalcq (1932) has shown by numerous experiments, in which eggs were cut in two in various planes, that formative substances of the egg of Ascidiella are definitely localized before fertilization in essentially the same patterns as in Styela and Ciona after fertilization.

Cerfontaine (1906) held that the Amphioxus egg was bilaterally symmetrical before fertilization, and I have found that the fertilized but unsegmented egg of Amphioxus has a pattern of localization essentially like that of the ascidian egg, namely, a mesodermal crescent around the posterior side, a chorda-neural crescent around the anterior side, an ectodermal area ventral to these crescents and an endodermal area dorsal to them. As in ascidian eggs, the first cleavage is always in the plane of bilateral symmetry and divides these crescents and areas into exactly equivalent halves, but the second cleavage, which is at right angles to the first, separates most of the chorda-neural crescent in front from the mesodermal crescent behind. Experiments prove that only the first two blastomeres are equipotential; lateral 1/2 or 2/4 blastomeres regulate by the peripheral substances of the two crescents flowing in over the originally median side

so that these isolated blastomeres become bilaterally symmetrical. The further development of such isolated blastomeres is typical, except for size. Isolated anterior or posterior blastomeres of the 4-cell stage, or any 1/4 blastomere, do not restore the formative substances that are lacking at the time of isolation and the further development of such blastomeres is more or less partial. Anterior blastomeres may form chorda and neural plate as well as a small amount of mesoderm and an atypical gut. Posterior blastomeres form an atypical gut and mesodermal somites, but no chorda or neural plate. Complete regulation occurs only in the restoration of bilateral symmetry in right or left 1/2 or 2/4 blastomeres. Except for this regulation, development of isolated blastomeres in Amphioxus, as in ascidians, is a mosaic work.

The development of partially separated and rotated blastomeres of the 2- and 4-cell stages of Amphioxus demonstrates that all the poles and axes of these blastomeres are irreversibly fixed as early as the first cleavage. All possible dislocations and rotations of such blastomeres take place in my experiments and the twin larvae that develop from these are united in corresponding ways. Usually anterior blastomeres of the 4-cell stage separate more readily than posterior ones and in the later development such blastomeres give rise to larvae double anteriorly and single posteriorly. But the antero-posterior axes of the two halves may vary from a condition in which they are nearly parallel to one in which they are at right angles to each other or even to a complete reversal of the chief axes in the two halves. Likewise the dorso-ventral axes in the two halves may run in the same direction or they may be completely reversed. All of these dislocations of embryonic axes are caused by the partial separation and rotation of blastomeres in the 2- or 4-cell stage.

Such dislocations and rotations of blastomeres of the 2- or 4-cell stage sometimes bring the ventral side of one

blastomere into contact with the dorsal side of the other, or the anterior side of one into contact with the posterior side of the other, but there is no indication that the neural plate and notochord of one act as an "organizer" in modifying the development of the other blastomere. In short, there is here no embryonic induction, such as Spemann and his associates have demonstrated in the case of amphibian embryos,—perhaps because each half of the Amphioxus egg contains its own organizer and the two halves develop synchronously. On the other hand, there is here the most convincing evidence of the early embryonic determination and self-differentiation of the blastomeres of Amphioxus.

In conclusion, the newer experimental work on embryonic determination indicates that in many cases where it was formerly thought that blastomeres were equipotential they are not really such. Localizations of different formative materials take place as early as the 8-cell stage in practically all animals, and in many forms such localizations take place as early as the first cleavage or even earlier. The earliest form of localization of different substances is more or less concentric, later a polar localization is clearly marked, and still later bilateral, anteroposterior and dorso-ventral localizations are evident. In a number of animal phyla all these localizations occur before the first cleavage, in others they appear only during the course of cleavage.

What is the formative agent in embryonic differentiation? Without attempting to find the *primum movens* we may conclude that if there are material differences in areas and cells it is not necessary to resort at once to some immaterial agent to account for their differentiation. It is impossible to understand, *i.e.*, to make intelligible, development except as a result of the formation and localization of different material substances. Indeed development consists in morphological division of substances and physiological division of labor. Cytologists and geneticists have made notable advances in the

study of the distributions of differentiated chromosomes and genes in the germ-cells, but the cytoplasm of the egg cell has frequently been regarded as mere foodstuff for these nuclear elements, in spite of the fact that practically all differentiation takes place in the cytoplasm. We are beginning to realize that the central problem of development lies in this relation between the genes and the cytoplasm, and that the cytoplasm is something more than mere nutritive "stuff."

Throughout my scientific life I have been waging a fight for the recognition of the importance of the cytoplasm of the egg. I was once introduced to a scientific audience as "the friend of the egg" when it had few friends, and I am now happy to find that its friends are increasing both in numbers and in importance. Fashions change in biology as well as in dress and they often move in cycles. If one only goes slow enough the rapidly moving procession may come up from behind and once more "the last may be first."

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ON THE ORIGIN AND DIFFERENTIATION OF THE SEXUAL GLAND[1]

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This discussion is to deal with the physiology of development of the sexual gland with particular reference to the processes of determination. The special topics to be considered are (a) the nature of the organization of the gonad-forming area at various stages of its early development, (b) the relationship of the primordial germ cells to the origination and differentiation of the gonad, and (c) the mechanism controlling the differentiation of the germ cell.

I

The evidence that the organization of the gonad-forming area of the chick varies from stage to stage has come from a study of the differentiation of the gonad-forming area in grafts to the chorio-allantoic membrane. In 1927 it was discovered in such grafts that the gonad rudiment of the genital ridge stage, although morphologically indifferent as to sex, is capable of undergoing self-differentiation into a gonad of specific sex. Invariably the right genital ridge differentiates into a testis or right ovary, and the left differentiates into a testis or left ovary. The rudiment is thus found to be specifically organized as to sex and in the case of the female as to laterality also (this conclusion as to lateral differences in organization is made possible by the well-known and striking differences in the structure of the right and left ovaries in birds). The genital ridge is, then, not physiologically but only morphologically indifferent as to sex.

These findings led quite naturally to an analysis of the potentialities of the gonad-forming areas of stages earlier than the genital ridge. These include (a) stages prior to

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the formation of the germinal epithelium, when the gonad material is prospective (donors having from 29 to 34 somites) and (b) stages at which a germinal epithelium is visibly differentiated, the earliest beginning of the gonad rudiment (donors having from 35 to 41 somites). In all these stages germ cells are present in the gonad-forming areas.

With respect to the differentiation of gonad, the results of this analysis are summarized briefly as follows:

(1) The gonad-forming area, just prior to as well as during the formation of the germinal epithelium, has the power to form a gonad of specific sex but with distinctly less frequency than the genital ridge (39 per cent. as against approximately 100 per cent. for the latter).

Furthermore, a gonad of specific sex differentiates from the gonad-forming area of a stage prior to the origin of the germinal epithelium in 20 per cent. of the grafts, whereas after its formation in 57 per cent. of the grafts.

It is thus quite evident that a gradual increase in the frequency of differentiation of a gonad of specific sex occurs as the morphogenesis of the gonad rudiment takes place.

(2) A gonad-like body of undetermined sex forms commonly (56 per cent.) in grafts of the gonad-forming area, whereas it rarely arises in grafts of the genital ridge. Prior to the 31-somite stage this is the only type of gonad thus far formed in eight grafts examined. Several grades of organization of these bodies are recognized, ranging from a mass of stroma containing few germ cells to a definitely circumscribed body consisting of stroma, sex cords of germinal and non-germinal cells.

(3) The gonad-forming area yields somewhat frequently multiple gonads, whereas a well-defined genital ridge gives a single gonad, as a rule.

(4) The size of the gonad of specific sex which arises from the gonad-forming area is smaller than one from the genital ridge.

It is thus seen that a progressive change in the developmental potentialities of the gonad-forming area occurs as it transforms into a genital ridge. The question now arises as to what this signifies. The gradual increase in developmental capacity is interpreted as indicating a progressive change of some sort in the organization of the gonad-forming area. That is, it is an index of an ascending organization, measuring apparently real differences in the specialization of the gonad-forming area at various stages in development. The gonad-forming area may have at first little or no specific organization as to sex. It merely possesses at the time of isolation an organization, conditioned by its position in the whole, which under favorable conditions in the graft may acquire the specific potentialities of sex by means of a series of processes. If, on the other hand, conditions (developmental mechanics, etc.) are so unfavorable as to interfere with morphogenetic processes, the harmonious whole of the implant and so forth, such a chain of processes may be halted at different stages, resulting in various grades of gonad-like bodies undetermined as to sex. In other words, the process of epigenetic development continues to a variable degree depending upon the developmental harmony within the implant.

Prior to the 31-somite stage the gonad-forming area may possess an organization of a lower grade still, since it had thus far failed in eight grafts to differentiate into a specific sexual gland, giving rise only to a gonad-like body. Similarly, Corinaldesi (1927), using a different method of analysis, was unable to obtain a gonad from the prospective gonad area of earlier stages.

The conclusion is therefore reached that the gonad-forming area during its initial development exhibits an ascending organization which finally attains at the genital ridge stage a fully determined condition.

II

The nature of the problem of the relationship of the primordial germ cells to the origin and differentiation of

the gonad may be pictured by two questions. Are primordial germ cells necessary for the origination of the gonad-forming area? Do germ cells have a stimulative (inductive) action on non-germinal cells and tissues, *viz.*, upon mesenchyme or coelomic epithelium?

Masses of germ cells may occur in abundance in the mesenchyme (or spaces within it) of grafts of the gonad-forming area, yet they fail to form a gonad or to cause with few exceptions a condensation of the surrounding mesenchyme. In such positions germ cells merely undergo repeated multiplication, exhibiting no "organizing power." A similar result, according to Dantschakoff (1932), is seen in grafts of the "germ-cell crescent" of Swift, grown in the extra-embryonic coelome of an early blastoderm or in the chorio-allantoic membrane, with the exception that in the latter a strong thickening of the mesenchyme is brought about. The apparent reaction of the mesenchyme to the germ cells is probably not specific, since a similar thickening is known to occur in response to non-germinal cells and tissues.

That germ cells may have an activating effect upon coelomic epithelium, however, is indicated. In a number of grafts of the gonad-forming area germ cells occur in abundance beneath the coelomic epithelium overlying the Wolffian duct or ostium of the Müllerian duct. This epithelium, thin and non-gonad forming in the normal embryo, thickens into a layer (possessing a few germ cells) which simulates strikingly a germinal epithelium. The underlying mesenchyme may even condense into a stroma, yet the essential components of an avian gonad, namely, the sexual cords, fail completely to invaginate.

On this problem the recent experiments reported by Dantschakoff (1931) have a bearing. According to her, following the destruction by electric cautery of the prospective gonad region lying just behind the omphalo-mesenteric arteries of the two-day chick embryo, the primordial germ cells ("die entodermalen Wanderzellen") settle down in more anterior levels of the splanchnic

mesoderm. Furthermore, when such "strange" splanchnic mesoderm, after it has reached an age of four or five days, is transplanted to the chorio-allantoic membrane it may give rise to a typical testis possessing germ cells. Since in all probability the gonad-forming areas normally extend anterior to the burned region, these experiments can not be regarded as demonstrating a stimulative action upon coelomic epithelium at a strange level.

On the contrary, the origin and differentiation of the germinal epithelium appear to be independent of primordial germ cells. This interpretation was first suggested by the formation of a testis with sterile sexual cords in grafts of the gonad-forming areas from 33- and 37-somite donor embryos. Since germ cells occur in the same grafts and consequently could have furnished the initial stimulus to the coelomic epithelium, this result can not be considered as decisive. The proof, however, of the independent origin and differentiation of the gonad—at least of the testis—has come from a study of grafts of whole blastoderms of early somite stages following the removal of the germ-cell crescent of Swift. In a number of such grafts a small testis has developed. In all these cases the sexual cords are typical in every respect, except that germ cells are entirely lacking.

In Amblystoma the reverse situation holds. Humphrey (1928) has presented evidence from graft studies that the germ cells must be aggregated in sufficient numbers next to or projecting into a coelomic cavity before a gonad forms. According to him the primordial germ cells play the dominant rôle in the early development of the gonad.

III

Whether or not a germ cell differentiates in the male or female direction depends apparently upon its tissue environment. In chorio-allantoic grafts of the gonad-forming areas two types of germ cells, differentiated and undifferentiated, are distinguishable. The former are

found in mesenchyme and in the medulla of the right and left ovaries; the latter occur in the sexual cords of the testis, cortical cords of the left ovary, and in certain gonad-like bodies. The undifferentiated cell is characterized by the presence of numerous dark-staining granules (presumably mitochondrial in nature) *uniformly* distributed in the cytoplasm. In the differentiated cell, on the other hand, the granules are *aggregated* into a group situated at one side of the nucleus. This difference in granular distribution in the cytoplasm of the germ cells appears to be an indicator of differentiation. Slight differences in the cellular differentiation of germ cells are seen in the two sexes. In the male sexual cord the granules of the germ cells are finer and lighter staining than they are in the female sex-cells of the cortex. Other differences are also noted. In the testicular cord the germ cells are large and occur singly, while in the cortex they are distinctly smaller and grouped.

The fact that the germ cells in the mesenchyme remain unchanged—*i.e.*, like the original germ cells, while those which come to be situated within sexual cords, either male or female, undergo structural changes—furnishes convincing evidence that they are dependent upon a specific tissue environment for their differentiation. The germ cell, although specialized in a general way as a sexual cell and thus differing from non-germinal cells, is really at first sexually indifferent or neutral and remains in this condition in such non-sexual tissues as mesenchyme. It undergoes specific cellular transformations as to sex only in the specific tissue environment of the sexual cord. In a testicular cord the transformation goes in the male direction, whereas in a cortical cord it goes in the female direction.

The apparent capacity of the indifferent germ cell to differentiate in either the male or female direction in the graft indicates that it possesses bisexual potentialities. This has been beautifully demonstrated in the female

fowl by an experimental study of the right ovary (see Domm, 1929). It has been found that if the left ovary is removed during the period when germ cells are still present in the right ovary, *viz.*, from the time of hatching until about the third week, it transforms into a testis containing male sex-cells; if removed after they have disappeared a sterile testis differentiates. It would appear from these experiments that (a) the germ cells remain indifferent in the medullary cords of the normal right ovary, and (b) when the inhibiting influence of the cortex of the left ovary is removed, the latent male potencies of the medullary cords become expressed in the form of seminiferous tubules, the germ cells therein undergoing a specific differentiation into male sex-cells and even into their definitive form, the spermatozoa.

Similarly in the amphibians Witschi (1929) has brought forward evidence of considerable weight that the germ cell is dependent upon a specific tissue for its differentiation into male and female sex-cells. According to his "theory of localized sex-differentiators" the sex-differentiating factor is localized in the cortex and medulla. The cortex is a female differentiating system, whereas the medulla is a male differentiating system. To put it in other words, the differentiation of the germ cell is determined by its location within the gonad.

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SOME DIFFICULTIES OF THE DETERMINATION PROBLEM[1]

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THE general title of this symposium leads me to comment on the origin and significance of the concept "determination" and to point out some of the difficulties encountered in its use. This word has come very much to the fore in the past few years, born, as it were, under an unlucky star and reaching maturity at a time when physics has nerved itself to cast aside the notion of causality in a strict sense and to talk indeterminism—to the comfort, I may add, of those wistful thinkers who see in this modern development of science a ground for their belief in the freedom of the will. It is as if a sort of Presbyterian biology were coming upon the scene just as physics is about to go over to the Baptists.

The preformationist embryology meant not merely that the qualities of the egg "determined" those of the completed organism, but even went so far as to assert that the organism was actually rolled up in the egg. So far as I am aware, however, the word "determination" was not generally applied in this sense. It, or its German equivalent "Bestimmung," came first into use in embryology in relation to the supposed action of internal and external factors in fixing certain qualities of the embryo, as, for instance, in Roux's (1883–87) studies "Über die Bestimmung der Hauptrichtungen des Froschembryo im Ei." Contrary to the general impression, Roux used the expression with reference to epigenetic as well as to preformational processes.

In fact, the theory of epigenesis, like the theory of preformation, postulates that the qualities of the egg lead to or determine the qualities of the organism that de-

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velops out of it, and that all the possibilities of development, presupposing of course a suitable environment, must be implied in some way in the egg. Inasmuch as this theory assumes that the localized qualities of the mature organism do not exist as such in the germ but arise gradually through the interaction of parts with one another and with the environment, that is through "Auslösung," or release of potencies, these factors are conceived as fixing or determining the qualities of other parts which are brought under their influence.

Though the expression was earlier used sporadically,[2] it was Korschelt and Heider (1902) who formally gave the name "determination" to this field of study. At the beginning of the chapter entitled "The Problem of Determination" they define it as follows (p. 81): "We count under this rubric the whole cycle of questions that deal with the disposition of the constituent parts of the embryo with reference to their future fate. Accordingly, it has to do with the origin, nature and localization of organ-forming factors—a field that embraces the fundamental questions of embryology and although already taken up from various sides, one that still remains very much in the dark." This was written in 1902. In 1932 I fear the subject still remains in the same condition.

The data of genetics and cytology show that the characters of the organism are represented in some orderly arrangement in the chromosomes. The results of experimental embryology indicate, though less conclusively, that the whole of the egg cytoplasm carries some features

[2] For example, by Wilson (1893, "Amphioxus and the Mosaic Theory," p. 617) and by Driesch (1894, "Analytische Theorie," p. 76). Conklin (1897) in his paper, "The Embryology of Crepidula," first proposed the phrase "determinate cleavage" to designate such types of cleavage as are found in mollusks, annelids, tunicates, etc., in which the pattern is definite and leads to the view that the history of each cell is predetermined (p. 190). The term "determinant," introduced by Weismann (1892), was used to denote a hypothetical unit of the germ plasm, resembling to some extent the "gene" of modern genetics. These determinants were supposed to fix or establish, in a purely preformationist sense, however, the qualities which later appear in the course of development of the organism.

that are fundamentally similar throughout and that the
several regions differ from one another mainly in the
relative intensity of their various activities. Accepting
these data, the general problem of development is how
such a system is transformed into one in which the vari-
ous parts differ more fundamentally according to region,
as they do in the mature organism. The problem is thus
in first instance one of segregation, as has recently been
emphasized by F. R. Lillie (1929). In other words, one
of the early important steps of development is the sepa-
ration locally of qualities that are at first combined or
mixed throughout the whole egg cell. It is what Driesch
called the distribution of potencies (die Verteilung der
Potenzen).

According to common usage, "determination" is mainly
applied to processes that occur after a certain amount of
segregation has taken place. Since it is in reality an in-
visible differentiation, it is essentially what Roux (1885)
had in mind when he expressed the opinion that the key
to the causal interpretation of embryonic development
lies in the answer to the question whether differentiation
takes place by itself (self-differentiation) or whether it
is the outcome of an interchange of effects between each
part and its surroundings (dependent differentiation).

This is a legitimate and satisfactory, if only prelimi-
nary, statement of problem which has led to many fruit-
ful experiments. Now it might all be well enough if we
confined our use of "determination" to the processes
themselves, and described the changes that take place as
differentiation proceeds, but trouble begins when, as is
more frequently done, we use the word to denote a state
and ask the question whether an organ rudiment is "de-
termined" or not, meaning thereby whether it is so fixed
as to its capacities that it can do but the one thing that it
does do. This difficulty rests upon the fact that there is
no certain criterion by which this can be answered. A
number of tests involving different conditions may be
applied, but they frequently do not give the same answer

to the question, nor do they tell us unequivocally whether the factor that is supposed to determine has ceased action or not.

The tests of determination may be classified roughly as negative and positive. Of the former, simple extirpation of the material from which an organ develops is the most readily applied. If, after removal of the rudiment, *e.g.*, that of the balancer or the lens in the amphibian embryo, the organ fails to develop, the rudiment is considered to be already determined. If the organ does develop after extirpation of the rudiment, the process is frequently spoken of as regeneration or postgeneration, but this does not preclude a considerable degree of "determination" or specialization in the primordium itself, for the same may in such cases often give rise to the proper organ when grafted to another place. The case is somewhat more complicated when, after extirpation, another piece of tissue is put into its place. Failure of the organ to develop does not then necessarily signify more than that the particular substitute lacks the power to form the organ, but the actual development of the organ means that the influences of surrounding tissues are still active. Such phenomena have led to the concept of "double assurance." Further knowledge of what is happening under such circumstances may be obtained, either by simple extirpation of the surroundings, more particularly the substrate, or by substituting for the normal surroundings something of a different nature.

The most usual positive test of determination is the performance of a given primordium when removed from its normal environment and placed in new relations in the embryo. In applying this test, however, differences are frequently shown between regions that are active in influencing what is near them and other regions that are more neutral. Ectoderm, taken from over the mandibular arch of the neurula or early tail bud stage in *Amblystoma punctatum*, gives rise to a balancer when transplanted to various parts of the head but will not do

so when grafted upon the trunk (Harrison, 1925). When taken in later stages, however, it gives rise to a balancer here also. Some continued action of the substrate seems necessary before the power to form a balancer becomes fixed in the ectoderm. An ear placode, taken at about the time of closure of the neural fold, develops into a highly differentiated and sometimes almost perfect ear when grafted between ear and eye, but it becomes only a small rounded vesicle without sensory areas when grafted to the limb region (Yntema, 1933). This difference can be due, if at all, only in part to mechanical factors, since ear placodes grafted at slightly later stages develop into much larger vesicles with well-defined sensory areas.

A more rigorous method of testing whether an organ rudiment is "determined" is by means of its powers of self-differentiation when planted out on a substratum, such as the chorio-allantoic membrane, that affords proper nourishment without exerting any specific morphogenetic stimulus. The most radical method is complete separation of the part to be tested from any other living material, as in the separation of blastomeres or in the explantation of the part under tissue culture conditions, but these methods also yield different answers to the same question.

A piece of medullary cord, taken prior to visible histological differentiation and isolated in a saline solution or in serum without contact with a solid substance, remains relatively inert, while a similar piece, placed in plasma, which later clots, differentiates into nerve fibers which extend far out into the medium (Harrison, 1914). The only difference here is a purely mechanical one, the chemical constitution of the medium being the same in the two cases. It is the presence or absence of a solid framework that decides whether the nerve fibers grow out or not.

The varying results in the above cases are due to different factors that are secondary to the processes of de-

termination. The effectiveness of such factors is brought out in an even more striking way by recent experiments made to test the self-differentiating powers of the various areas of the amphibian gastrula and early blastula.

These experiments have been carried out by Kusche (1929) and by Bautzmann (1929b) independently by implanting small pieces of the Triton embryo into the enucleated eye socket of a young larva, and by Holtfreter (1931), who explanted similar pieces to the body cavity or to a saline medium *in vitro*. The results are astonishing. First there are striking differences, depending upon the method of isolation used, especially in the ability of presumptive ectoderm to give rise to nervous tissue, which, according to Holtfreter, takes place in the body cavity but not in the saline medium. Secondly, all three investigators agree that what Bautzmann has termed "bedeutungsfremde Selbstdifferenzierung" occurs. For example, notochord and mesoderm may differentiate out of pieces that would ordinarily give rise to neural plate; nervous system may arise from presumptive abdominal ectoderm and ectoderm from presumptive neural plate. Pieces taken from the region of the organizer, if relatively large, tend to develop into complex bodies, consisting of foregut, notochord and mesoderm or of notochord, mesoderm and nervous tissue, according to the exact location from which they are taken in the early gastrula stage, but smaller pieces tend to differentiate into pure notochord, muscle or a vesicle of foregut epithelium. In general, according to Bautzmann, the region of the organizer is characterized by equality between its prospective potency and its prospective significance, although Holtfreter does find that it can give rise to nervous tissue, as well as to notochord, mesoderm and foregut epithelium. From these experiments one can not fail to conclude that the isolated part tends to form a greater variety of structures than when left in place in the embryo. Its diverse potencies may thus be realized

without the influence of an organizer acting from without.

Let us consider now certain invertebrate embryos in which determination is said to take place very early—during cleavage or even before. For instance, in the ascidian egg, as Conklin (1905, 1931) has shown, the fate of the cells in early cleavage is already determined in accordance with the segregation pattern of the cytoplasm in the unsegmented egg, which becomes fixed during maturation and fertilization. Disturbances in the arrangement of the cytoplasmic masses or of the blastomeres, produced by strong centrifuging, by alteration of the cleavage planes or by injury to single blastomeres, result in corresponding abnormalities in the mature larva. According to Dalcq (1932), the future constituents of the embryo are localized, in part at least, even in the unfertilized egg, before the final arrangement of cytoplasmic substances is reached. These are facts well established by varied and convincing experiments from which one would naturally infer that the adult ascidian would have little or no power of regulation. Yet what does the study of asexual reproduction and regeneration teach?

It has long been known that certain organs, such as the ganglion and the walls of the peribranchial space, do not originate in the bud in the same manner as in the embryozooid. In the matter of regeneration Driesch (1902) showed that in Clavelina complete and perfect restitution takes place in either direction from a cut surface at practically any level. Even a fragment of the highly differentiated branchial basket may regenerate a whole organism, or the branchial basket itself may be reproduced by the visceral sac. In the latter case, according to Brien (1930), the pharynx and the peribranchial space grow out from the epicardial tube, itself a derivative of the pharyngeal endoderm, and the ganglion is budded off from the wall of the right peribranchial cavity. This agrees, in some measure at least, with the process of budding in

some forms,[3] but in Clavelina, according to the most recent account of P. and E. Brien-Gavage (1927), budding takes place in the stolon in quite a different manner. Here the stolon septum is mesenchymatic and is not derived from the endodermal epicardial tube, as has been generally maintained, and the whole bud, except its epithelial covering of ectoderm, is formed out of this mesenchymatic tissue, supplemented perhaps by wandering elements from the blood. Furthermore, in Clavelina, under adverse conditions (in winter in northern waters and in spring and summer in the Mediterranean) the ascidiozooids undergo reduction[4] and in the stolon buds mesenchyme cells gather in immense numbers, forming loose aggregations inside the ectodermal membrane. Out of these buds new zooids are reconstituted when external conditions become favorable. Spek (1927), who has made a thorough study of the question, emphasizes the rôle played by certain vacuolated cells (Tropfenzellen), but P. and E. Brien-Gavage find that the morphogenetic function of reconstituting new zooids falls to ordinary mesenchyme cells, while the vacuolated cells are nutritive. Finally, in certain cases isolated branchial baskets may undergo reduction and reconstitution, as Driesch described, during which process the highly differentiated cells are destroyed and only mesenchyme (including vacuolated) cells remain inside the ectodermal sac; out of these a new zooid is formed, as in the case of the winter bud.

While some of the processes just described are not altogether clear, particularly, whether dedifferentiation and redifferentiation or actual destruction of tissues, followed by restitution out of totipotent reserve cells, takes place, it is absolutely certain that in budding and reconstitution totipotent mesenchyme cells play the prin-

[3] Of the numerous papers on the budding of ascidians the following may be cited in this connection: Hjort (1895), Caullery (1895), Ritter (1896), Lefevre (1898), Seeliger (1900–06).

[4] See papers by Giard et Caullery (1896), Kerb (1908), Schaxel (1914), Huxley (1926), Spek (1927), Salfi (1927).

cipal rôle. The segregation pattern found in ontogeny can therefore have but temporary significance. The blastomeres may be "determined," but if so, only for a time, for endoderm later has the power to form the nervous system and the peribranchial cavity, while mesenchyme has the potency to form all tissues except possibly ectodermal epithelium.

The question raised here is an old one and has been fully considered by Weismann (1892) and others in connection with regeneration. It is not sufficiently taken into account, however, in relation to embryonic development. The facts just presented go to show that the general qualities of the organism must persist in cells that may seem to have had their properties restricted to a more specific rôle, and that other capacities than those for which a cell seems to be determined in early ontogeny may be called forth under proper conditions.

Let us now leave the passive side of the process, the "being determined," and consider some of the active factors which are described as determining. One of the earliest recognized examples of these is the optic cup in relation to the lens, as originally described by Spemann (1901) in *Rana fusca*. If the eye rudiment is removed from the medullary plate before contact with the skin is established, the lens, which is normally formed from the latter, does not develop—a typical case, as we should say, of dependent differentiation. However, in closely related forms, *R. esculenta,* for instance, this does not occur (Spemann, 1907, 1912). After precisely the same operation the lens does develop, in some cases very perfectly, and it is concluded that determination takes place here at an earlier period, in which case, however, it must be some agent other than the eye rudiment that is active.[5]

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[5] The results of von Ubisch (1925) tend to minimize the above contrast, for he finds that in *R. fusca* imperfect lenses may arise and in *R. esculenta* they are not fully differentiated. Moreover, external conditions such as temperature, salinity of medium and uncontrollable individual differences seem to affect the results.

The positive test, first applied by Lewis (1904) in *R. sylvatica* and *R. palustris* and since confirmed for many other species, brings out in a more striking way the influence of the eye upon the lens. In embryos with open neural folds or with primary optic vesicle ectoderm taken from other regions is grafted so that it will lie over the eye in place of the lens ectoderm which is removed, or else the optic vesicle is implanted under the ectoderm of other regions. As a consequence, lenses, in many cases quite perfect, develop out of the overlying strange ectoderm. In *Bombinator pachypus* and perhaps in some other species there are regional differences in the ectoderm at the period marked by the close of neurulation, for ectoderm taken then from the head develops into a lens, whereas that from the trunk does not (Spemann, 1912).

One of the most baffling results came from the following experiment. If the optic vesicle is removed from the embryo of *Amblystoma punctatum* just after closure of the neural folds, the lens, as Le Cron (1907) found, does not differentiate out of the ectoderm which remains. However, take this same ectoderm away from the optic cup and transplant it to the region of the ear or the heart, instead of taking the optic cup away from it, and it develops into a perfect lens quite independently of any eye (Harrison, 1920). It is impossible, therefore, to say that the optic vesicle determines the lens or that the latter at the period concerned is determined in any absolute sense. We are justified only in speaking of what it does when certain conditions are imposed.

The emphasis upon "determiner" and "determined" leads to a very lop-sided and often erroneous view of the process, for it is questionable whether one factor can influence another without being itself changed. This becomes obvious when we inquire into the influence of the lens rudiment upon the eye. Little is known of the early activities, but later very marked effects in this direction are shown. In the absence of the lens the vitreous body is defective, the eye does not properly expand, and the

retina may become folded (Beckwith, 1927). In later stages the mutual relations between lens and eye in respect to growth rate are clearly brought out by heteroplastic transplantation of either lens epithelium or optic vesicle alone between *Amblystoma punctatum* and *A. tigrinum* (Harrison, 1929). These two species show enormous differences in their respective growth rates and in the ultimate size to which the individual grows. When a whole eye is grafted from tigrinum to punctatum it may attain double the linear measurements of the normal eye of the host. Likewise, when the lens epithelium alone is grafted, a lens that is at first too large for the eye is formed, but later an adjustment between the two takes place. The growth rate of the eye is accelerated beyond that of the normal and the growth of the lens is correspondingly retarded, until the organ, as a whole, comes to an equilibrium at a point intermediate between the extremes of the two kinds of eye and with eye-lens proportions approximately, though usually not quite, normal; but the influence of the lens upon the eye is quite as great as that of the eye upon the lens.

The most important advance in embryology of late years has been Spemann's (1918, 1924) discovery of the organization center and organizer in the amphibian egg. This is localized in a definite region, as a cytoplasmic differentiation which may be identified with the gray crescent in the unsegmented egg and later with the dorsal and lateral sectors of the border zone between the animal and the vegetative portions of the egg. It is turned in during gastrulation to form the roof of the archenteron, and afterward differentiated into pharyngeal roof, notochord and mesoderm. Its powers are extraordinary, and almost anything brought into its neighborhood is made in some mysterious way to cooperate in forming an embryo of a varying degree of completeness. It has some limitations—its capacity for self-differentiation is restricted (p. 311) and its powers of organization wane as differentiation proceeds—but at first these powers are

of cyclonic proportions, carrying away in a puff our preconceived notions of preformation and germ layers. They last sufficiently long to mould the whole embryonic axis and fix the various regions of the future nervous system, and are then transferred to the medullary plate itself, which thus acquires the capacity to incite by homogenetic induction the formation of a new medullary plate when grafted under presumptive ectoderm in the blastula or early gastrula (Mangold and Spemann, 1927; Mangold, 1929). Nothing is known about the reciprocal effect of the organized material upon the organizer, but I venture to predict that when this is studied it will be found not to be negligible.

The use of the term ''organizer'' is likely to be attended by some confusion, for the word may be readily taken to imply more than we are really justified in attributing to the thing itself. The material upon which the organizer acts is already highly organized. The explantation experiments referred to above show that it has the capacity for diversified self-differentiation. The organizer, itself a complex system with different regional capacities, merely activates or releases certain possible qualities which the material acted upon already possesses. The orderly arrangement which results depends not only upon the topography of the organizer but also upon that of the system with which it reacts (Bautzmann, 1929 and 1932; Spemann, 1931).

Besides the main organization center there exist subsidiary organizers, the general action of which is shown by the experiment first thought out (Gedankenexperiment) by Spemann (1921), and actually executed by Schotté (Spemann and Schotté, 1932; Schotté, 1932). Indifferent abdominal ectoderm of an anuran is placed over the mouth region of a urodele embryo. To the underlying cells of this region the powers of a secondary organizer are ascribed, since a mouth and suckers, indeed almost a face, are actually formed out of the grafted tissue, but it is an anuran mouth and not that of a urodele.

As Spemann is reported to have put it, the ectoderm says to the organizer, "you tell me to make a mouth; all right, I'll do so, but I can't make your kind of a mouth; I can only make my own and I'll do that."[6] This raises a difficult question—the significance to be attached to the abstraction mouth. Though radically different morphologically and histologically, the two mouths must have something in common, for the organizer of the one, by exerting what Spemann and Schotté term a "komplexer Situationsreiz," calls forth the other out of material that would ordinarily produce something else.

What it is desired again to emphasize is that the material which becomes "organized" merely develops certain of its own qualities in interaction with surrounding factors. Other conditions may draw out other qualities. The same kind of ectoderm that produces the mouth may, as Spemann (1918) has shown, give rise to nervous tissue if grafted into the medullary plate, where it would come under the influence of a different organizer. It is therefore said to be still undetermined in the early gastrula, but this statement must be taken with the reservation that the material is not entirely indifferent, since, if isolated, as in Holtfreter's experiments, it differentiates into nervous system and epidermis. The material thus retains not only the general characters of the species but also a variety of different special potencies, some of which it may still hold long after determination in a particular direction may seem, according to some criteria, to have taken place. There is no way of finding out with certainty whether the particular quality which a cell seems to have is finally fixed, for there always may be new conditions, not yet tested, under which other potencies might be revealed (*cf*. Schleip, 1929, p. 878). Recognition that intermediate conditions may persist for a time has led to the use of qualifying terms, such as labile, reversible or partial determination, but such phrases are in fact self-contradictory and are of little real use be-

[6] I ask Professor Spemann's indulgence for making use of this hearsay quotation.

cause not precisely definable. It is false to regard such conditions as exceptional and as requiring special explanations. They belong in reality among the most essential characteristics of developing organisms and deserve a place in our philosophy of development commensurate with their importance.

I have elsewhere (Harrison, 1921) laid stress upon the fact that differentiation takes place gradually through a series of small steps. Some of the stages of this process have been ascertained in the case of paired organs, such as the limb and the ear, but there is probably no one who would maintain that the steps in the determination or differentiation of the lens are the same as those in the case of the limb. To classify both in the same terminology does nothing to clarify our understanding of what goes on.

Experimental embryology will be placed on a sounder basis if its questions are framed more carefully. In dealing with such a complex system as the developing embryo it is futile to inquire whether a certain organ rudiment is "determined" and whether some particular feature of its surroundings, to the exclusion of others, "determines" it. A score of different factors may be involved and their effects most intricately interwoven. In order to resolve this tangle we have to inquire into the manner in which the system under consideration reacts with other parts of the embryo at successive stages of development and under as great a variety of experimental conditions as is possible to impose. Success will be measured by the simplicity, precision and completeness of our descriptions rather than by a specious facility in ascribing causes to particular events. There is always room for fallacy, even when the logical procedure may seem unimpeachable, and no conclusion in embryology is safe if based upon but a single proof. This, to some, may all seem purely formal and of no practical consequence. It is, nevertheless, important to realize that even the language of science is still bound by tradition (*cf.* Hogben, 1931) and is by no means free from anthropomorphisms

and relics of our demonology, which are difficult to escape and which may not only lend a false sense of security to our explanations but also may suggest foolish questions that never can be answered.

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FUNCTIONAL ADAPTATION AND THE RÔLE OF GROUND SUBSTANCES IN DEVELOPMENT[1]

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WHEN it was first known from the work of Culmann and Meyer that the arrangement of the lamellae in the spongy bones was such as to correspond to the trajectories of tension and pressure acting on the bone, the idea of a direct influence of mechanical function on the organization of the tissues seemed to be substantiated. Even more so, since Wolff (1892) could demonstrate that the internal structure of bones subjected to abnormal strains, as in pathological cases, changes profoundly into a pattern corresponding to the new situation and supposedly adapted to it. This is what Roux (1895) then, called "functional adaptation." Later, similar phenomena have been observed also in the cartilage and in the connective tissue systems (Benninghoff, 1931). Although these phenomena were found in the adult, Roux was ready to proclaim adaptation to the functional requirements of activity as a general principle in morphogenesis. There was, however, one essential point utterly opposed to such an attempt; that is, that the so-called functional constructions, in the embryo, already exist long before there is any corresponding functional activity. So, although there are functional structures, there is certainly nothing of the kind of a functional adaptation, in the original sense, to be found in the embryo. Rhumbler (1914), later, demonstrated that the internal architecture of the embryonic bone could be explained on the basis of assuming that internal tensions arising from the differential surface growth of the bone are the organizing factors. This system of growth tensions incidentally coincides, in its main features, with the tensions

[1] Paper read at the symposium on "Embryonic Determination," before the American Society of Zoologists, Atlantic City, December 30, 1932.

that would be created by an external load, and the so-called functional structures are rather growth structures. This idea has later been corroborated by Triepel (1922), Benninghoff (1925), and others. All these authors still consider tensions to be the agents in the formation of the so-called functional structures, differing from Roux only in that they attribute these tensions to growth instead of to external load.

In a further attempt to approach, experimentally, the problem of functional structures, I started, a few years ago, a series of experiments, the results of which may be outlined as follows: The purpose was to produce functional structures in tissue grown *in vitro* outside the organism; in other words, to imitate in the tissue culture, the conditions within the organism. So, I devised a method by means of which it was possible to grow fibrocytes in a medium subjected to appropriate tension (Weiss, 1928, 1929). A thin membrane of blood plasma was coagulated in a tiny horizontal glass frame and a fibrocyte culture was put in the center. The distribution of tensions in the membrane is a definite function of the geometrical form of the frame. In the case of a triangular frame, for instance, the maximum tensions are directed towards the sides of the triangle. Under these conditions, the direction of the outgrowing cells coincides with the lines of maximum tension. In a triangular frame, we see the cells deviating from the radial direction and converging into three main bunches, directed, as are the principal tensions, toward the sides of the frame. Varied experiments of this kind always yielded essentially the same results. As in the functional structures of the organism, the cells, in their arrangement, duplicate the pattern of tension trajectories. Similar results have since been described by Huzella (1929). I further found that not only the orientation but also the intensity of cell growth is influenced in that the growth[2] proceeded at a higher rate in the directions of stronger tensions.

[2] "Growth" of a tissue culture includes both multiplication and outward migration of the cells.

Considering these experiments, one might be led to believe that they furnish decisive proof for the direct action of mechanical factors on the direction and intensity of cell growth. This direct action could be either an immediate pull or some kind of trophic stimulus in the sense of Roux. Nothing of this sort, however, holds true. It can easily be realized that there is no way in which pull could act directly on the cell. There is, on the one hand,

the coagulated medium in equilibrium with the tensional forces to which it has been subjected, and there are, on the other hand, cells freely traveling in or on it. It is obvious that these traveling cells, themselves, are as little aware of, and affected by, the tension existing in their support as a man lying on the ground is aware of, and extended by, the tension existing in the earth's crust. Unfortunately, this simple fact is sometimes entirely overlooked, so that we find in the literature effects described as tension effects in cases where tension obviously was not in play at all. Furthermore, some of our experiments bear striking evidence against the view that

tension might have acted directly on the cells. If the culture medium, having been coagulated under the influence of tension, is removed from the frame and put on a support so as to release it from the original tensions, we observe, in favorable cases, the cells nevertheless growing out in those directions which mark the former tension trajectories. Tension, therefore, can not be the immediate agent influencing the cells. We found in the experiments the cells following lines of tension which had already ceased to exist. In order to account for this result, we have to assume, first, that tension had had some orienting effect on the medium in such a way as to establish therein a system of material lines coinciding with the tension trajectories, and that these lines, then, formed pathways over which cells might migrate. The second inference is that such pathways really are preferential traffic lines for the cells, no matter whether tension persists or not.

It has been suggested (L. Loeb) and proved (Harrison, Burrows, Carrel) that tissue cells in order to be able to grow and to migrate require some solid structure as support (Harrison, 1914). In demanding a supporting substratum these cells behave like amoebae. We can not, however, enter here into a discussion of the various explanations which have been suggested to account for this peculiar behavior of cells. Terms like "thigmotaxis," although not elucidating the situation, may still be accepted for the sake of description. Probably surface tension relations on the liquid-solid interfaces along which the protoplasm extends play an important rôle in determining the thigmotactic movements. Hence, inasmuch as different kinds of protoplasm are likely to have different surface tensions, we may expect to find given interfacial structures serving as leading structures for one kind of cell while not affecting another kind at all. In any case, it is a fact that the fibrocytes in the tissue culture tend to use liquid-solid interfaces as tracks, the solid phase being represented as fibrin, the liquid phase

as serum. If, now, the particles of the solid phase are
arranged in a definite orientation, the movement of the
cells will necessarily be correspondingly oriented.   Thus,
the problem of the trajectorial arrangement of the cells
reduces itself to the problem of a trajectorial arrange-
ment of the fibrin particles.   And remembering now that
particles in a colloidal substratum, as has long been
known, can be oriented by mechanical tension (V. Ebner,
1906), we can replace the idea of a direct action of ten-
sion on cell growth by such a conception as is represented
in the following schematic picture.   The ultramicrones
or "micellae," as we may call them, using the term of
Naegeli, are, in the case of organic colloids, generally of a
definite polarity which in itself may be an expression of
the constitution and arrangement of the high molecular
compounds composing them.   We may think of these
micellae as being little rods of sub-microscopic dimen-
sions (Schmidt, 1924).   In a fresh colloidal sol, these
little rods are irregularly distributed.   Without the in-
tervention of polarizing agents, they will aggregate to
form fibrillar threads, but there will be no definite orien-
tation in the resulting network.   If, however, there is
some vector force, for instance tension, acting on the
system, the rods will all be oriented in such a way that
their polar axes nearly coincide with the direction of that
force (Ambronn and Frey, 1926).   Usually, from the
further agglomeration of polarized and oriented par-
ticles, oriented fibrillar structures result.   As Baitsell
(1915) and others were able to observe, fibrin fibers in a
plasma clot subjected to tension attain even microscopic
visibility.   It has to be mentioned, however, that in the
case of my experiments, besides a lamellar structure, no
coarse fibrillation of microscopic dimensions could be de-
tected, from which we may conclude that as a guiding
structure for the cells an ultramicroscopic orientation is
sufficient.   We will call such an ultramicroscopic struc-
ture "ultrastructure."   In our frame cultures, for ex-
ample, the tensional forces acting in three main direc-

tions cause the formation of a correspondingly oriented metastructure in the clotting medium, and the outgrowing cells simply follow the preestablished pattern. However, as I stated above, not only the orientation but also the growth rate of the cells was affected. As a matter of fact, the particular ultrastructure of the medium can account for this phenomenon, too, since the growth rate depends upon the supply of liquid nutritional substances, and the distribution, circulation and supply of liquid with respect to the growing culture prove to be profoundly influenced by the ultrastructure. The displacement of liquids is facilitated along the lines of micellar orientation. Hence, growth, too, is favored along these lines.

The reason for having reported these experiments at some length is not only that they apparently furnish some clue as to the mechanism involved in the development of functional structures. Their main significance seems to lie in that they unmistakably point toward a more general principle of morphogenesis of which the so-called functional constructions are only special cases. This general principle, which may be called "principle of ultrastructural organization," as applied to the organism, could be formulated as follows: The interior architecture of the body, as expressed by the arrangement of cells and intercellular formations, is to a certain extent determined by the ultrastructural organization of the colloidal continuum which fills the interior of the organism.

Before, however, being entitled to apply to the organism this principle derived from the somewhat artificial conditions of tissue culture, one has to prove that, in the essential points concerned, the conditions in the organism and in the tissue culture are strictly comparable. The best example to prove the comparability is the process of regeneration of tendons. Tendons show the most pronounced functional structure since their cells and fibers are all oriented in the direction of pull.

From surgical experience as well as from experiments of Levy (1904), it can be learned that, after cutting a tendon, the gap between the stumps is soon bridged again by true tendinous tissue with the typical lengthwise arrangement of fibers and cells, provided a longitudinal tensional strain has been allowed to work on the regenerating tissue. In the absence of the oriented pull, the regenerated tissue does not show any oriented structure. The situation is essentially the same as in the tissue culture. There is, at first, a clot of blood formed which connects the stumps. This clot is, then, subjected to tensional stress resulting in a lengthwise orientation of the micellae, and all the immigrating cells, eventually, follow the preestablished oriented pathways. Even more striking perhaps are experiments of Nageotte (1922). He implanted in the connective tissue of adult animals pieces of tendons which had previously been fixed in alcohol, thus offering to the cells a medium with a preformed structure; and, really, cells which happened to penetrate from the surroundings into the dead graft attained, therein, such arrangement as is typical for tendons. Undoubtedly experiments of this sort bear more than a purely superficial resemblance to the tissue culture experiments. The resemblance is even closer if we take into consideration that blood plasma in the organism (Nageotte, 1931) as well as in the tissue culture (Maximow, 1929, Baitsell, 1917) is able to develop true collagenous and argyrophile fibers. Mentioning them, it must be added that they are by no means to be identified with what we consider to be the general guiding structures of cells. Where fibers are differentiated they undoubtedly can be utilized as tracks by the migrating cells. For the rest, ultramicroscopic ''ultrastructures'' do as well.

It is, of course, obvious that the medium in which the connective tissue normally arises in the embryo is not blood plasma, as in the case of tissue culture or wound healing; however, in all features that concern organiza-

tion it is similar to blood plasma, similar especially in that its micellae are polarized and hence capable of being oriented along the lines of force. This medium is known in later stages as the ground substance of the various organs. It appears in earlier stages as the ground substance of the mesenchyme and can even be traced back to still earlier, pre-mesenchymal, stages as mesostroma, filling the spaces between the germ layers (Snessarew, 1932). And it may be that there is some substance present even in the unsegmented egg playing an analogous rôle. At least, the way in which Lillie (1909) speaks of the "ground substance" of the egg suggests this possibility. There is still much discussion about the origin and nature of the ground substance. Some believe that there is no ground substance which is not part of living protoplasm, and some others believe that ground substances are only dead secretions of cells, like mucus. Between the two extremes, we find all intermediate shades of opinion. It is not unlikely that some time it will come to be realized that the facts are such as to preclude a uniform solution of the problem. There is one thing, however, to be considered as certain. That is, that the properties on which the formation of ultrastructures is based exist in every protoplasm. We find them at work in the processes of mitosis, in the formation of intracellular tonofibrillae, myofibrillae, neurofibrillae, etc. But it is equally certain that substances which are not protoplasmic, or no longer protoplasmic, as, for instance, some body fluids, possess in that one respect the same properties. For that reason, there is, from the viewpoint of the present problem no need of further discussing the question of whether the ground substances originate as detached ectoplasmic substances or as transformations of protoplasm or as cell secretions of some kind. We may simply record one significant point in which all the different opinions are in accord, namely, that the ground substances form a continuous system all through the embryo, from the earliest stages on. This proves

that a material which is suitable to ultrastructural organization and comparable to the medium of tissue culture is present in embryogenesis.

As to the further assumption that cell arrangement depends upon structures in the ground substance, normal embryogenesis also furnishes appropriate examples. The chorda sheath,[3] the cornea and the vitreous body of the eye, for instance, at first, consist only of a cell-free colloidal layer into which cells later immigrate, proceeding along the preestablished pattern of the ground-substance. Therefore, one has to admit that, in the points under consideration, conditions in the tissue culture and in the embryo are strictly comparable.[4]

Now, however, in the embryo, what are the forces which shape the ground substances, which bring about such ultrastructural organization as has been artificially induced by mechanical factors in our *in vitro* experiments? Are the factors in the embryo mechanical as well? It has already been mentioned above that many of the organ structures considered as functional structures are merely growth structures. They are caused by internal tensions resulting from the surface growth of the organ. In these cases, tension is undoubtedly the factor which impresses an oriented structure upon the ground substance. Wherever this is the case, the particular pattern of tension trajectories is determined by the peculiar form of the growing surface. This latter plays a rôle similar to that of the frame in our experiments. If, in the simplest case, the contour of the growing organ is circular, a radial structure is to be expected. That is exactly what we find in the tympanic membrane of the ear. As the

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[3] V. v. Ebner, *Zeitschr. f. Zool.*, 62, 1896.—The chorda sheath shows a very marked "functional structure." *Cf.* Tretjakoff, *Zeitschr. f. Zellforschg. u. mikr. Anat.*, 4: 266, 1927.

[4] Harrison was probably the first to emphasize this similarity; in 1914 (*Jour. Exp. Zool.*, 17, 521) he states: ". . . since it has been shown that most embryonic cells are stereotropic, and that such arrangements as they assume in the embryo may often be induced under cultural conditions by reactions to solids, there is a presumption in favor of the view that this type of reaction is a potent factor in normal development also."

tensions, exerted on this membrane in post-embryonic life by sound waves, happen to coincide with the embryonic growth tensions, the final structure of the organ looks as if it were functional by origin (Benninghoff, 1931). A more irregular contour causes a more complicated pattern of tension trajectories. For this Benninghoff (1931a) brings a very instructive example. After implanting the dead scapula of a human fetus under the skin of a rabbit, he observed the formation of a fibrous capsule around the graft. The arrangement of the connective tissue of this capsule was very similar to the fiber pattern found in normal scapular periosteum. During the normal development, the tensions determining the orientation of fibers arise from the growth of the scapula; in the experiment, they arise from the contraction of the connective tissue which coats the graft. Since, however, the distribution of tensions is determined by the form of the contour, and the contour being in both cases the same, the pattern of tension trajectories and, hence, the pattern of fibers is similar in both cases, too. We are confronted here with one of the simplest manifestations of a "Gestalt" principle, since we are dealing with a system of typical configuration in which the arrangement of every part is strictly dependent upon the whole. The external shape of the organ is not brought about by mere apposition of elementary parts in a definite arrangement, but, on the contrary, the arrangement of the parts, *i.e.*, the internal structure, is determined by the shape of the entire system. This is further evidenced in cases where an organ, after having undergone some pathological alteration during morphogenesis, shows internal structures which are entirely different from the normal, but which fully correspond to the new shape the organ as a whole has assumed. Cases of this kind have been described in chick embryos with malformed bones by Landauer (1929).

Like growth, the mere change in the shape of an organ will, of course, alter the existing tensions and occasion-

ally establish new ones. Furthermore, it is obvious that the growth, or change in form, of an organ not only affects the ultrastructure inside but affects the surrounding ground-substances as well. Imagine, for instance, the growth of a vesicular or tubular organ. It is easy to realize that by increasing in diameter it must create a steady tangential tension in the surrounding medium which, eventually, leads to the formation of concentric fibrous capsules and sheaths. A tubular organ growing in length and width is bound to exhibit both longitudinal and circular tensions on the surroundings which may result in a corresponding orientation of the surrounding tissues. Examples are found everywhere in the body.[5] Carey (1922) has called attention to the fact that the arrangement of muscle tracts in the embryonic limb can be explained on the basis of tensional stresses exerted on the surrounding mesenchyme by the differential growth of the skeleton. A similar explanation was claimed for the arrangement of the muscular layers in the intestine (Carey, 1921). Considering all existing evidence, it seems that this idea is perfectly substantiated, as far as merely the spatial arrangement of the tissue is concerned. On the other hand, there has not yet been offered any convincing proof to show that the differentiation into muscle tissue is likewise determined by tensional strain. On the contrary, whereas there is some indication that stretch of some kind is essential for the maintenance, and maybe also for the differentiation of muscle fibers, the facts revealed by experimental embryology carry sufficient evidence to emphasize that the factors determining a cell to transform into a myoblast are of a more specific nature than mechanical stress can ever be.

From what we have stated above, it becomes clear that even the epithelial forerunners of organ formation, by

[5] It is obvious that the tangential stretch which leads to the formation of a fibrous capsule around an organ is not always produced by increase in size of the organ, but may as well be due to a gradual contraction of the surrounding substance, as is the case in the formation of capsules around foreign bodies.

their moving, folding, stretching, swelling, and so on, must have a marked effect on the ultrastructure of the underlying ground substance, in later stages on the mesenchyme. It is however, hard to say whether this phenomenon is of significance in the process of organ formation or is just a transitory incident. Observations on the development of glands by Flint (1903) suggest that the epithelial formations may play some rôle in the organization of their supporting or surrounding stroma.

The fact that I have been dealing at some length with the effects of mechanical tension may give a wrong impression about the real share which those tensions take in organization. As a matter of fact we frequently find their importance overrated by many authors. Of course, we do not know very much about the other orienting forces besides tension which intervene in morphogenesis. But we know, at least, a little, and this may be outlined as follows: One non-tensional factor which can be assumed as certain to exhibit organizing activities by stamping ultrastructures in the ground-substance is the displacement of fluids, both by slow diffusion and by faster circulation. You all remember having seen brooks streaming over grass or weeds. You remember how the blades were oriented by the current, as if combed. This gives an illustration of how the circulation of liquids in the ground-substance causes the formation of an ultrastructure oriented along the stream lines. Steady diffusion potentials work, of course, in the same way. Undoubtedly, many of the whorl-like formations in organisms can be explained on this basis. I have frequently observed typical stream-lined structures in the semi-fluid regeneration blastema. The morphogenetic action of the displacement of fluids is, of course, most pronounced in those cases where there is excessive resorption of water during growth. Triepel (1911) was already aware of the fact that the fiber tracts of the connective tissue in a tadpole's tail mark those directions in which water from outside penetrated into the tail, and those along which it

shifted therein. Once aware of these factors one will
probably detect them in many places. It is, for instance,
very likely that a difference in the water content of dif-
ferent organs has effects of the kind described. To a
certain extent, I was able to reproduce and analyze even
in tissue culture the directive effect of fluid displacement.
A growing tissue culture, by sucking fluid from the sur-
rounding medium, establishes a steady diffusion gradient.
If, now, two cultures are put in the same medium, their
reciprocal suction will result in a movement of liquids
along their line of connection. This movement results
in the formation of a corresponding ultrastructure
bridging the two growth centers, and the bridge becomes
visible, as soon as the cells grow over it (Weiss, 1929).
In the embryo, pictures of this kind, showing strands of
cells forming straight connections between two centers
of high formative activity are a familiar occurrence. If
there is a mechanical barrier somewhere in the ground
substance, currents from the surroundings will be de-
viated in a direction parallel to the wall. This causes
the formation of stream-lined ultrastructures which
sometimes can be easily mistaken for tensional struc-
tures. The transverse growth of a tendon regenerated
along a silk thread inserted at right angles to the direc-
tion of the tendon stump, as found by Levy (1904), is
apparently due rather to the flow of liquids along the
thread than to tension, as the author was inclined to be-
lieve.

Another factor which during morphogenesis un-
doubtedly is involved in the formation of ultrastructures
is the electric field. Electric fields may act either directly
by orienting the polarized micellae or indirectly by caus-
ing cataphoretic effects. These latter consist in the es-
tablishment, in the capillary spaces, of an oriented mi-
gration of electrically charged particles along the lines
of electric force, and this polar migration has, of course,
the same orienting effect on the ground substance as a
streaming fluid. Increased chemical activity in a circum-

scribed area leads to structural effects both by causing diffusion streams and electric potential differences.   The chemical activity may furthermore alter the qualities of the ground substance itself, may cause dehydration and fibrillation or, on the other hand, by means of proteolytic enzymes, cause some liquefaction and destruction of existing structures.   And if we try to survey all possibilities, the situation becomes so intricate that, at least, one obvious objection against our conception, namely, that it might be too simple, certainly can not be upheld.

As an example of how the principle of structural organization can be applied to special problems in embryology, I may briefly outline its explanatory value for the problem of nerve patterns, both peripheral and intracentral.   As Harrison (1910) has concluded from his tissue culture experiments, outgrowing nerve fibers show a similar affinity to solid structures, as do mesenchyme cells.   So, there was good reason to believe that ultrastructures in the ground substance might be responsible for the orientation of the growth of the nerve fiber.   This assumption is substantiated by the observation of Held (1909) who saw the first processes of the embryonic neuroblast extend along the extremely fine filaments of the mesostroma connecting medullary tube, myotomes, notochord and ectoderm.   Thus, the facts support the idea that the immediate factors in the orientation of the nerve fiber are correspondingly oriented guiding structures. On the other hand, however, various other kinds of factors have been proved to influence the orientation of nerve fiber growth.   The theory of neurotropism as a form of chemotaxis has been advanced by Cajal,[6] electric fields have been claimed by Kappers (1927), Child (1921) and Ingvar (1920) and Detwiler (1926), finally, have clearly shown the attractive influence on the outgrowing nerves which is exerted by a developing organ rudiment. The principle of ultrastructural organization offers a common and uniform explanation for all these cases, by

[6] *Cf.* Tello, F. Vortr. u. Aufs. u. Entwicklungsmech. H. 33, 1923.

assuming that neither the chemical nor the electrical
agents act directly on the nerve fiber, but that both, pri-
marily, produce ultrastructures in the ground substance
which, secondarily, serve as guiding paths for the nerves;
chemical centers by diffusion currents, electric centers
by cataphoresis, both resulting in structures converging
toward the respective centers. The occurrence of a periph-
eral nerve plexus in the limb region could be explained
by assuming that there is some metastructural barrier
arising from the meeting of two fields of activity, one be-
ing the developing limb bud, the other being the axial
organs of the embryo. An interlacing of nerve fibers,
corresponding to what in the organism occurs as plexus
formation, could also be observed in the tissue culture in
places where there was some sudden change in the
mechanical condition of the medium.[7] The formation of
intracentral fiber tracts may obey similar rules. Ac-
cording to Kappers, Coghill (1929) and others, central
fiber tracts always connect such parts of the brain as dif-
ferentiate simultaneously. This reminds us exactly of
the conditions of the experiment described above where
a cell bridge connected two growing cultures. Further-
more, Bok's (1929) demonstration of the dependence of
the internal architecture of the brain upon its curvature
almost forces us to consider this architecture as trajec-
torial. These few remarks may suffice to show the ap-
plicability of the principle.

Having established and demonstrated the principle of
ultrastructural organization its general bearings may
now be pointed out. It is clear, at first glance, that the
principle tends to replace a number of different agents
acting at a distance which have been hitherto considered
as orienting and organizing factors. If cells arrange
themselves in definite directions this should not be ex-
plained any longer by specific attractions nor by trophic
stimulation, but by the establishment of oriented path-
ways in the ground substances, these latter being the

[7] P. Weiss—Unpublished results.

common playground of all kinds of forces released during development.[8] Although the principle apparently applies only to moving cells, leaving the cause of their displacement unexplained, a consideration of the changes in surface tension relations, in distribution of liquids, etc., may possibly lead to an understanding of this latter point, too. A certain difficulty seems to arise, however, from cases where in one and the same organ or embryonic district one part shows definite orientation, while another part does not, or does, but differently; for instance, nerves running across muscles. It is, however, possible to account also for these cases if one keeps in mind two things: First, a possible specificity of the pathways; and second, what we may call the time factor. What specificity of the pathways means was expressed above when we said that differences in the surface tension relations at the solid-liquid interfaces of the medium may well explain why one type of protoplasm extends along the interfaces, whereas another does not. As to the time factor, the rôle of which in development has been especially emphasized by Goldschmidt (1927) and by Brandt (1928), we must be aware of the fact that the directive agents during embryogenesis are very unstable, most of them acting only temporarily, during a definite period. A center of higher developmental activity will, of course, act as an organizing agent on the ground substance only as long as its activity lasts. As soon as its activity decreases and other areas rise to increased activity, the previous ultrastructural pattern will gradually be replaced by a new pattern. Structures developed on the basis of the new pattern will, then, no longer show relations to the structures originated at an earlier time. In this respect the principle agrees with the general conception of tem-

[8] There is evidence to show that not only the orientation and arrangement but also the shape of the cells is materially influenced by the organization of the ground substance. It might, therefore, well be that in the loose connective tissue the lamellar structure is not, as Laguesse believes (Arch. de Biol. 31, 173, 1921), the result of, but is the cause for the flat shape of the cells contained therein.

porary gradients of activity as conceived by Child (1929).
Taking into account the typical sequence of developmental processes, the time pattern, so to speak, we recognize the developed organism as being, in some way,
comparable to a photographic plate on which different pictures have been developed successively, and the difficulty
mentioned above ceases to exist. One more thing, however, has to be assumed. That is, that the ultrastructures
are plastic and labile enough to give way to new ones, if
this is required by the situation. This point is the main
reason why so much emphasis has to be laid on proclaiming ultrastructures and not manifest fibrillar structures
as the general guiding principle. Ultrastructures, indeed, are plastic and reversible and thus conform to the
requirements of embryogenesis; fibrillar structures, generally, are not. Fibrillar structures, too, are not altogether unmodifiable, but their breaking down seems to
be too slow to keep pace with the rush of developmental
changes. Since, however, ultrastructures tend to transform steadily into coarse fibrillar structures, provided
they are not stirred up by continual interferences, a
gradual loss of plasticity during development may be expected. The degree of plasticity found in any part of
the embryo will, then, essentially depend upon how long
an organizing factor has had time to act on the surroundings. After a certain duration of continuous uniform
action, the ultrastructure will be found to be almost irreversibly transformed into a manifest structure no
longer susceptible to new influences; such solidified structures, however, will, on the other hand, be able to serve,
henceforth, according to their previously attained organization, as a typical guiding system, even if the original
organizing factors do not persist or have changed in
character. We will say of such a system that it has undergone ''irreversible determination'' and has changed
from dependent differentiation to self-differentiation.
This remark should, however, not be mistaken. I do not
think we can expect that the general problem of deter-

mination could at some time be reduced to so simple a basis. Past experience has warned us of being too prompt in generalizing. So, instead of proclaiming the principle of ultrastructural organization as a universal clue for the problems of embryonic organization, we may content ourselves, for the time being, with the recognition that it is *one* of the principles or mechanisms of morphogenesis. Its best feature is that it opens innumerable ways for further experimental attack.

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MUTATION AND ADAPTATION AS COMPONENT PARTS OF A UNIVERSAL PRINCIPLE:

III. THE SPECTRUM OF LIFE

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IN the preceding sections of this essay, as we have trained our eyes and sharpened our focus, it has become increasingly evident that just as there is a solar spectrum that reflects the details of intra- and interatomic activities of the matter constituting the sun, if we knew fully how to register and interpret it, so there appears to be a spectrum of life, which an omniscient eye could read and interpret. Moreover, we were able to see and interpret portions of this spectrum. In this section we wish to extend our consideration of this spectrum of life.

In the latter part of the preceding section a suggestive analysis of the autocatalysis cycles of a man was made. The spectrum could not be fully described or interpreted owing to our ignorance. Such a spectrum would correspond to a single "octave" in the whole spectrum of man's existence.

A similar spectrum involving longer cycles might be prepared for the race, if we knew enough of its history and its destiny to fill it in. Although we can not do this, we can briefly discuss a few ideas that would arise in such a spectrum.

When we attempt to visualize events in the life of an individual or of a nation, in terms of a succession of periods of adaptation and mutation we get a comprehensive unification of familiar phenomena, that seem to be more or less unrelated as we usually view them. This can be well illustrated by the history of two recent mechanical inventions. Many people can recall when the bicycle was first introduced and can trace its subsequent history. There was a fore-period in which the mechanism and manufacture were perfected and in which abid-

ing interest, as distinguished from idle curiosity, grew.
Then came the period of rapid growth and wide-spread
utilization, as manufacturing methods became economical
and efficient. This was followed by a period of slacken-
ing of interest in which the bicycle ceased to arouse curi-
osity and in which it became a toy or a strictly utilitarian
means of locomotion. Meanwhile the first automobiles
were making their appearance. They too passed through
a fore-period of growth and manufacture. We have re-
cently passed into the period of maximum growth of
interest in and of manufacture of automobiles in this
country and presently we shall pass into the last phase,
into the period of complete practical adjustment in our
use of this means of locomotion. Meanwhile, the aero-
plane has come along. The development and maturation
of this spectacular means of locomotion was prematurely
hastened by the needs and interests of the Great War.
Its problems are not yet fully solved and it is probably
not yet known just what it may contribute permanently
to man's help and comfort.

In a similar way, we could perhaps show that the his-
tory of every innovation, produced in the development of
man, follows a similar course. The use of fire, the build-
ing of roads, the use of horses for travel, the development
of stage coaches, of railroads, of plows, of looms, of type-
writers, of telephones, and of hundreds of other comforts,
utilities and practises of human life, past and present,
have developed in a similar way. This even extends to
the styles of clothing that we wear and to the minutiæ of
our personal habits. It is almost trite to say so because
we are so accustomed to it, but there is always the fore-
period, the period of maximum interest or change and
the after-period of loss of interest.

When we analyze the history of developments of any
kind we find that they all show a fore-period of prepara-
tion followed by another period of maximum activity
succeeded by another period of rest and preparation for
the next cycle. There is some kind of external stimulus

and internal accommodation that constitutes the fore-period; this is followed by a period of great activity until depletion in one of the necessary agents occurs, and then the process enters into the decay stage which may be the fore-period of a new cycle. The dimensions of a given cycle must be retained in discussing that cycle. Cycles within cycles occur here also to an almost unlimited degree of complexity. Within its own dimensions the characteristics of a given cycle are not disturbed by another or series of others of a larger or smaller scale. We shall discuss these things more fully below.

Growth of human knowledge an autocatalyzed process: Viewed in broad general terms the phenomena that we have just been discussing may be considered as education. Both the development of the innovation or invention in the minds of men and its transmission to others are autocatalyzed processes. We know when we come to think of it that education follows the autocatalytic curve. We find that our effective interest in any subject is slow of growth. It then comes to full maturity and intensity, and finally wanes unless fed by new view-points and material. Achievement is likewise autocatalytic. Men finish their work in one field and move to another if they wish to remain fruitful. Wilhelm Ostwald in his study of the biographies of great scientists, undertaken in order to learn the secret of their greatness, concluded that this slow initiation of achievement and gradual shifting of interest is a rather general trait of the greatest fruitfulness of creative genius and cites Helmholtz as a notable example. This he suggests may be due to the exhaustion of certain materials in some of the neurones, that are essential to the development of the autocatalysts, while in other neurones an unimpaired stock of these materials is still available, but unfortunately not transferable. But it is also possible that the fruitfulness of genius depends upon the presence of raw material, so to speak, in the environment. Just as an amoeba fails to grow in an environment containing no food, so genius may fail to grow

in the absence of its own peculiar "food." The nature of this "food," or stimulus to fruitfulness, varies with the nature of genius. And so it comes about that a change of activity or of surroundings acts like a renewal of youth. Men of genius are wise to use one or both of these methods to sustain their productivity.

As we grow older we learn to recognize this sort of fatigue or exhaustion for what it is and to discount the negative reactions that sometimes accompany it. It is so much more difficult to recognize it in great men, who have performed heavy labors, that it is beyond our ability to comprehend. T. B. Robertson in the preface to one of his books[1] describes an interesting instance of this sort. Bergson, in his "Creative Evolution," asserted his belief that the scientific method can never ascertain a solution of the riddle of development. Robertson's comment is in part as follows:

This assertion affords but one among very many contemporary instances of waning faith in the power of intellect; a reaction, perhaps against its overwhelming successes, which nevertheless continue without ceasing, and augment. . . . So little effort and we are now exhausted! So brief a period and we abandon the conflict! We declare ourselves impotent, because in a day we have not comprehended the ultimate significance of life! . . . M. Bergson has himself eloquently and convincingly dwelt upon the vast import of our understanding, and the argument which he advances is applicable no less to the more intangible advances of theoretical knowledge. Each successive acquirement enlarges our power to grasp more, and the progress of human knowledge is in fact autocatalysed.

We must pause here and emphasize the fact that the autocatalytic growth of knowledge either in the mind of man or in the tradition of the race is not simpler than an autocatalyzed chemical reaction, where the proper autocatalyst is not the sole prerequisite. There must be a suitable substrate, a suitable medium and a suitable energy environment. Failure of any of these will make the autocatalyst ineffective. As educated men and as educators we generally fail to appreciate either in ourselves

[1] "The Chemical Basis of Growth and Senescence," Lippincott Company, 1923.

or in others the complexity of the processes that we are trying to set up.

If the workings of the mind and if the growth of knowledge, in the individual as well as in the race, are indeed autocatalyzed processes, as we have been saying and as many think, then we as educated men have a very deep and personal lesson to learn, both in courage for ourselves and in tolerance for others. When we have finally by a slow autocatalyzed process reached the burning conviction that we are in the possession of new or valuable truth we must have the courage and patience to set up and keep going the autocatalyzed process of learning in our colleagues, until they too see the truth of our new knowledge.

In this connection we are reminded of the familiar story of Mendel. One of his friends sympathized with him twenty years after the publication of his famous work on heredity in sweet peas, because his work was not yet recognized. Mendel comforted his friend by saying, "My time will come." He knew of the long lag in learning, of the slow growth of the autocatalyst involved.

Autocatalysis in history: In recent years certain scholarly historians have pointed out the similarity of the curve representing the development, fruition and decline of races, of civilizations, of political institutions, etc., and the autocatalytic curve. In doing this they have been led to a rather pessimistic outlook from a consideration of the last phase of the simple autocatalysis curve, *i.e.*, the period of depletion of the substrate. When the autocatalysis is considered as a unit, as a finished thing or process, the last phase of a cycle constitutes a very unsatisfactory stage. Because of the great progress or change taking place in the preceding phase it comes to be viewed as a dénouement period, the "sucked-lemon" period. It is my opinion that these writers have carried the physico-chemical analogy too far or else not far enough. They have failed to note that any autocatalysis curve of a chemical process represents only a segment of

the life history of the bit of matter in question. As we have seen above, a suitable change is pretty likely to occur that will give rise to a new cycle and so the disappointing part of the curve becomes the fore-period of a new cycle.[2] Of course the new cycle can not be the same cycle as the one just past. Moreover, in terms of the duration of human life, it may be that we can not live to see the next cycle completed, but we need not for that reason fall into tears by the shores of ''time,'' thinking that life is of no further interest because the last breaker has rolled in. It is true that for us it may be the last of its kind, but we may be comforted because even as we watch it finish its course the forces of the ''sea'' are being accumulated as the fore-period of the next great wave. And so it seems to be with the ''breakers'' or cycles of history.

The ancients had no such grounds for comfort in this respect as we have. If we had stood with the people of Greece and had seen that beautiful wave of democratic civilization break on the shores of time and slip back into the seas we might have wept for grief and inability to see the new great wave of democratic civilization in the state of formation. We, however, happen to live in a time in which another great wave is emerging. Perhaps none of us can yet say whether this cycle of progress in democracy will sweep the world or whether even now a greater broader wave is forming. We know that the democracy of Greece was made possible by slave labor. In the cycle now approaching completion slavery has largely been replaced by mechanical power. The age of machinery has raised the crest of the wave to a higher

[2] I first encountered such ideas somewhere in the historical writings of Henry Adams. Later of course in full force in Oswald Spengler's ''The Decline of the West'' and also in K. C. Schneider's ''Die Periodizität des Lebens und der Kultur'' (1926). It is beyond my capacity to add anything to the discussion of the problem of the cyclic nature of history beyond the simple suggestion of developing a more unified view and of seeing things in ''scale.'' The work of those writers would therefore constitute authoritative discussions of certain cycles or even ''octaves'' of the great spectrum of life that we are trying to visualize.

level than it had attained before. Without going into further detail it is already evident that the present cycle is vaster in its scope, since it aims to include all men and not just a selected group. It is therefore quite unlike the former one and if it fails to sweep the world we shall have other grounds for disappointment than those experienced by the Greeks. The two could not be interchanged in time.

These cycles of progress have their proper order and sequence and this is the ordered sequence of musical notes in their octaves and that of spectral series. Many historians have had an intuitive sense of this ordered quality of the continuity of history, and this quality is distorted by a restricted use of the autocatalysis curve. To view the various aspects of civilization, or the process as a whole as an autocatalyzed process is apparently justified, but to leave out the principle of continuity by emphasizing the single cycle gives rise to pessimism. Such a pessimism contradicts our intuitive sense of continuity, of progressive evolution. This unreasoned and unreasoning sense of moving forward that possesses us is satisfied to work and rest alternately, as is provided for in this spectral picture of life and history.

Many writers have more or less intuitively realized this peculiar progressive continuity. Recently George Sarton expressed it as follows:

> During the course of my studies of human progress, I have often had the impression that everything happens as if mankind was working in shifts. The accomplishment of its essential task is so hard that periods of creation are often followed by periods of fallowness.[3]

Autocatalysis of the evolution of Man: If we consider only the lower portion of the autocatalysis curve we have in a clearly recognizable form a so-called hysteresis curve, *i.e.*, there is a lag behind the calculated response, when we are looking for a linear response. Without attempting to review or discuss hysteresis in its usual ap-

[3] "Introduction to the History of Science," Vol. I, p. 443, Williams and Wilkins.

plication, we may at once consider what may be called the hysteresis of history, but which we now see to be the autocatalysis of the evolution of man.

In the course of man's evolution the most interesting and unique activity in which he has indulged is his comprehensive attempt to conquer his environment. Certainly there are but few living organisms that have made achievements comparable with those of man in this respect. Insects have much to their credit, in their adaptation of materials to their needs, but theirs is no longer a progressive tradition, for a given species, as in the case of man. The earliest knowledge that we have of man's conquest of his environment depends on the elaboration of crude stone implements. By the presence of such relics the relative age of human remains can be determined. By those relics it is indicated that perhaps a half million years ago man had made more progress in a voluntary conquest of his environment than any mammal has made up to the present. There is no way of knowing how these men discovered that by attaching a stone to the end of a club they could strengthen the power of their arms, but it is clear that such an initial step as this was more significant in man's development than all that follows. Such was the great new idea, the germ, so to speak, of a new autocatalyst that was to transform the whole nature of man and his attitude toward the universe.

It has been suggested that it took perhaps four hundred thousand years for this new idea to grow and ripen enough so that man made marked improvements upon these crude beginnings and then the Paleolithic period of his history becomes the Neolithic period, according to our terminology. During this long dark period opposition to the acceptance of the "new fangled" ways of fighting, with hammers, for instance, was only slowly overcome as tribes saw themselves decimated because of their unwillingness to accept and use stone hammers. By Neolithic time innovations took place more readily and man was using this new conscious way of improving

his life with more facility. Handicraft had a high place
in social esteem, because it represents a skilful harness-
ing of the brain and the hand. Because of improved
interest and skill progress during this period was more
and more rapid so that after perhaps another one hun-
dred thousand years we come to the invention of the
steam engine. For some time before this man had been
approaching a new type of conquest—a conquest of
power—and the steam engine represented the fruition of
this movement. With this invention a new age of still
more rapid progress, in the conquest of our environment,
was ushered in and it is in this age of headlong advance
in which we are now living.

If we attempt to make a diagram representing the
above facts and plot time against the amount of change or
progress it is evident that we have the fore-period and
the first part of the fruition period of the autocatalysis
curve. If we pause now to consider what the remainder
of the fruition period and the final or completion stage
may bring to man we are silent. It is not likely that any
one can predict in broad terms what the remainder of
man's history will be, when viewed as a whole in these
terms. If we view history as an autocatalytic process it
follows that the change or progress occurs as the result
and in answer to some external tension or stimulus. But
the system in turn must contain within it the capacity to
respond. What can be the nature of this stimulus and
what can be the latent form of the response contained
within the system. We are quite accustomed to visualiz-
ing some aspects of the two halves of this situation in the
simpler forms in which it occurs in physics and chemis-
try, but here these pictures are inadequate. We must
remain satisfied with a general picture. We see this
earth of ours and all that it contains lying like a mass of
molecular dimensions in an energy field of unknown qual-
ities, so far as their final effect and influence are con-
cerned. We feel a kind of cosmic heat warming us up
toward efforts of development and achievement in which

the first steps at least are taken in darkness and uncertainty. When shall we achieve a clearer sight in these fields? Or is the scientific method in some degree the organ of sight for the mind as the eye is the organ of sight for the body?

At first it seemed that this is the longest autocatalysis in the known history of man that we could put our fingers on. When, however, we remember that man in his individual development undergoes a physical, intellectual and spiritual development and that the length of the autocatalysis curves for these increases in length in the order mentioned, the possibility of a longer cycle of development becomes evident. The evolutionary development of man's physical nature lies in the unknown past, the development of his intellectual nature as a race is in the state of active development. Where is the evolutionary development of his spiritual nature as a race? If the analogy holds will it not have a longer "wave length" in our historical or evolutionary spectrum than either his physical or intellectual evolution? Could we not expect that the emergence of man's intellectual nature should be accompanied by the early stages of his spiritual development? When we think of it in this way we get a clearer insight into the significance of man's persistent interest in his own origin and destiny and in his ultimate relation to the universe.

Such vague speculations and yearnings are usually lumped together and called religion. We see sporadic leaders of such interests rise and fall; they have experienced some power and insight in themselves and have tried to develop the autocatalyst in their neighbors and the record shows us that they have done so with measurable success. Consequently, during the past four or five thousand years there has been an observable increase of interest and of effective usefulness of a spiritual autocatalyst. However, even the most optimistic could scarcely assert that man has as a species entered into the period of full fruition in this strange realm. But none

will gainsay, if we have read the record correctly, that if and when man does enter into the period of fruition of this long autocatalyzed process, in which at best he is still in the fore-period or period of preparation of the autocatalyst, he will undergo as strange and marvelous a transformation as that of the savage carrying a club with a rock tied on the end of it, being transformed into the highest product of man's present development. In respect to the development of his religious or spiritual nature man is somewhere in the Paleolithic period of his development. The present strife and struggle among the various forms of ethical religion represents the growth and perfection of the autocatalyst, an attempt to pass from the Paleolithic to the Neolithic stage of spiritual development.

Résumé: In this section we have extended the description of phenomena that follow the "S" curve resembling the autocatalytic curve. We have always found the "adaptation" associated with a "mutation." We have seen that when one cycle of this sort succeeds another they are different in some respect. We found such auto-catalyses to be linked and interwoven, just as the various "wave lengths" of light are interwoven in sunlight. Although we could have elaborated the description of this "spectrum of life" in much greater detail and could have cited many other processes that follow this course, nothing significant would be contributed in this way. We are now interested to see, in the remaining section of this essay, if it is possible to find any basis upon which the behavior of such diversified systems can be visualized so as to give rise to this "S" curve. We can not hope to explain the long chain of events, but it may be possible, so to speak, to string them, as it were, on one connecting thread.

THE GENERAL BEARINGS OF RECENT RESEARCH IN OENOTHERA[1]

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THE first period of genetic research was largely devoted to a rapid accumulation of data regarding many plants and animals by a relatively few investigators. The domestic or semi-domestic animals and plants particularly came in for early examination, many of the classical cases of Mendelian inheritance in rats and mice, fowls, sheep and cattle, maize, wheat, Lathyrus, Primula, Antirrhinum and Capsella dating from this period. Bateson, with his colleagues, became the world leader in the study of Mendelian behavior.

Already, however, in the pre-Mendelian era, the genius of de Vries had developed methods of pedigree breeding and amassed a large body of what we should now call genetic results. The genetics of Oenothera thus began a decade or two earlier than that of other plants, with the exception of Mendel's original experiments with garden peas. In the last two decades, under the guidance of Morgan, Drosophila has loomed large in animal genetics and has served for the elucidation of such fundamental discoveries as the correspondence between the number of linkage groups and the haploid number of chromosomes. When this principle was confirmed by Punnett for Lathyrus it clearly became of general validity. The phenomena of non-disjunction, trisomics and polyploids also appeared incidentally in Drosophila. Thus it has come about that Oenothera among plants, and Drosophila among animals, have longer and more complicated genetic histories than any other organisms.

Apart from the fact that cytological and genetical observations and principles have become more and more intimately interwoven throughout the whole period since

[1] Paper presented at the Sixth International Congress of Genetics, Ithaca, N. Y., August 29, 1932.

the beginning of the century, another conspicuous movement in recent years has been the phylogenetic study of particular genera, especially of plants, on a cytogenetic basis. Sumner's investigations of Peromyscus, its geographic sub-species and varieties, are a notable case on the animal side, in which the principles of evolution rather than the actual phylogeny were mainly considered. Thus, after a period in which genetical investigators were almost inclined to look askance at the discussion of evolutionary problems, the experimental study of phylogeny and evolution, through the investigation of the chromosomes, the hereditary genes and the systematic relationships of a group of forms, has come to the fore in various genera such as Nicotiana, Crepis and Triticum. By such means will genetics come into closer touch with the general problems of evolution. It seems clear that in the near future many intensive evolutionary investigations will be carried out involving the combined and co-ordinated study of the cytology, genetics, bionomics and geographical distribution of a circumscribed group and leading to a fuller understanding not only of the lines along which their phylogeny has taken place but also of the environmental conditions and the internal developmental factors which have been most effective in connection with their evolution. At the present time cytology is initiating such studies in many groups by comparative investigations of their chromosome numbers and morphology.

In Oenothera much has now been done which can contribute from many angles to a study of the phylogeny of the genus. I propose to refer to this subject later, but I wish first to make brief reference to the earlier genetic history of Oenothera. It is so well known that I need scarcely remind you that the first work on mutational changes in chromosome numbers was done with Oenothera. The classic case of *Oenothera gigas* was the first genetic tetraploid, with which the vast modern study of polyploidy began. Similarly, the first trisomic was *Oenothera lata,* and the first triploid *O. semigigas.*

Again, in *O. rubrinervis* the first case of non-disjunction
in any organism was discovered. These developments
take us up to 1915.[2] They provided an explanation of the
manner of origin of many of the well-known mutations
originally described in the experiments of de Vries; and
cases of similar character are now known in many other
genera of plants and animals. Curiously enough, how-
ever, although polyploidy and to some extent non-dis-
junction are undoubtedly of phylogenetic significance in
connection with the evolution of many plant genera and
families, yet they have apparently played no part in
Oenothera, where they were discovered, since all the
known wild species of Oenothera agree in having 14
chromosomes. Evidently in this genus some other proc-
ess has been at work, and we may expect to find it
in connection with the chromosome catenation which is so
wide-spread in the genus.

In the post-war period the study of fixed chromosome
linkages or catenation began with the paper of Cleland
(1922) on *O. franciscana*. This has now become a field
of very active research, but many uncertain interpreta-
tions are involved, some of which will be discussed later.
We now know that the great majority of species, hybrids
and mutations of Oenothera show more or less complete
catenation of their chromosomes during the period imme-
diately preceding the heterotypic metaphase in which
chromosome reduction takes place. But the condition,
which appeared for some time to be unique in Oenothera,
is now known to occur also, in Rhoeo, Datura, Pisum,
Aucuba, Campanula, Humulus, Avena, Zea, Rosa, Pole-
monium, Hypericum, Rumex, Briza and Anthoxanthum,
which are representatives of a series of Moncot and
Dicot families. But only in Rhoeo and Hypericum has
the complete catenation which is so characteristic of
Oenothera been found.

That catenation has played an important part in the
evolution of the genus seems clear, but we have at pres-
ent no means of knowing why it has become so para-

2 Gates, ''The Mutation Factor in Evolution.''

mount in Oenothera while in many other genera it has little or no significance. I think, however, we may understand the situation better when we realize that catenation helps to perpetuate a heterozygous condition, and that the concomitant hybrid vigor will give a marked selective advantage to catenated over uncatenated forms. It has been shown experimentally that a ring of 4 or a ring of 6 can arise by crossing two homozygous Oenotheras each having 7 free pairs of chromosomes. In the presence of balanced lethals the catenated hybrid form will persist, while the homozygous recombinations will entirely fail to appear; but even without such lethals there is every evidence that the more homozygous recombinations with less catenation will fail to survive under natural selection because of their markedly decreased hybrid vigor. Thus the catenation of chromosomes, once begun in a hybrid form, would tend to increase to the maximum—a ring of 14. The forms with less catenation, such as half-mutants like *O. rubrinervis,* which may be supposed to arise through segmental interchange between chromosomes of a ring belonging to different complexes, will have less hybrid vigor, and may therefore be expected to be snuffed out in competition with the more heterozygous forms from which they are derived. The same will be true generally of completely homozygous segregates such as *deserens* and *blandina.* Thus hybridization appears to be a necessity for the development of maximum catenation such as Oenothera shows, and in this respect it must have played a fundamental rôle in the evolution of the genus.

Elsewhere I have discussed (Gates, 1933) the phylogeny of the genus Oenothera mainly from the systematic and geographical points of view. Here it is only necessary to consider the various genetic principles involved. Investigators of the genus are agreed that the large-flowered southern species are primitive, and it seems clear that these were at first confined to Central America and Mexico. As the ice retreated at the end of the Pleistocene they moved northwards and gradually spread

over the continent. Since they have no special method of seed dispersal, it is reasonable to suppose that their spread was slow and that there was ample time for evolutionary differentiation to take place in localized areas.

Now there appears to be a definite relation in Oenothera between flower-size and latitude, the small-flowered species being massed in the higher latitudes while the large-flowered forms are found mainly in the South. There is considerable evidence (chiefly unpublished, see Gates, 1932) that the species with small petals have been derived by a series of dominant mutations from those with larger flowers as this northward movement was taking place. It seems that genes for smaller flower-size have appeared independently in different lines of descent, *e.g.*, on the Atlantic and the Pacific coasts. In general, decrease in length of style goes hand in hand with diminishing flower-size, so that the large-flowered species have long styles and are open-pollinated while the small-flowered species are usually self-pollinated. Although there are exceptions, it is quite probable that the same gene which decreases the length of petal has a similar effect on other parts of the flower, including length of style. Statistical evidence derived from large numbers of measurements of petal length in various interspecific Oenothera hybrids leads to the conclusion that some of the small-flowered species, but not all, contain several genetic factors for decrement in petal length. It is also found that these size genes are not all of equal value, some producing a larger effect than others. Further evidence is furnished by the fact that in *O. novae-scotiae,* a small-flowered species with complete catenation, one of the complexes contains factors for a considerably larger flower-size than the phenotype of the species (see Gates and Catcheside, 1932).

Since decreasing flower-size leads to self-pollination, it is probable that hybridization was of more importance and of more frequent occurrence in the early history of the genus than in its later development. The bionomics of the small-flowered species are altered in several other

respects. Having smaller flowers, they save considerable energy in the production of calyx and corolla. The anthers are also much shorter and produce a relatively small quantity of pollen—again a considerable saving of energy. Further, notwithstanding the smaller quantity of pollen, they are much more efficient seed-producers, as any one knows who has grown the small-flowered and large-flowered species side by side. The former will usually show a complete setting of capsules filled with seeds, because the stigma of every flower is already pollinated before it opens (except at the end of the season), while in the large-flowered forms many flowers have failed of pollination through lack of insect visits, and the amount of seed production is usually much less. As a seed-producing mechanism the small-flowered species therefore have a marked advantage over those with large flowers; and since they are permanent crypthybrids owing to the chromosome catenation, they do not suffer from homozygosis although they are generally inbred to the maximum degree. All these considerations help us to understand why there is a general diminution of flower-size with increasing latitude, and why the forms with smaller flowers occur generally in the areas most recently occupied in the northward migration. It would be interesting to know whether genes for flower-size have played a similar rôle in any other genus of flowering plants.

If we consider now the evolutionary factors which have been concerned in the phylogeny of the genus Oenothera, four at least may be mentioned: (1) gene mutation, (2) hybridization, already considered in certain aspects, (3) catenation, (4) segmental interchange of chromosomes. With the exception of (1), these factors or conditions are intimately associated with each other. It appears clear that gene mutations are as necessary to account for the evolution of Oenothera as of other plant and animal genera, but it would appear that they are usually masked by the existence of complexes, which in turn seem to depend upon catenation of the chromosomes.

The known gene mutations are *brevistylis* and genes for dwarfing discovered by de Vries in *O. Lamarckiana* and *O. gigas; rubricalyx,* the only known dominant mutation; and those which Shull has been particularly successful in obtaining from *Lamarckiana,* namely, *supplena, vetaurea* and *bullata* (Shull, 1925, 1926, 1928); also *acutifolia* recently described by Brittingham (1931).

The fact that, according to Shull, these fall into three separate linkage groups in a species having only one free pair of chromosomes, is difficult to reconcile with the apparent absence of gene mutations from other species with high catenation. If *O. Lamarckiana* can show unlinked gene mutations, why do not other related species having high catenation show free Mendelian segregation when crossed? The evidence that they do not is so clear, both from double reciprocal hybrids and from other systems of breeding, that we are forced to conclude that linkage of chromosomes is involved in their failure to appear. Shull endeavors to solve the difficulty, so far as *Lamarckiana* is concerned, by assuming that the position of the chromosomes in a ring is not fixed in the sense that chromosomes of each complex always alternate with each other. He assumes that for each pair the arrangement in the ring may be AB or BA, which would give the effect of free segregation for each pair which had this degree of freedom. There are serious difficulties with such a hypothesis in *Lamarckiana* itself, but the difficulties in explaining thus the hybrid behavior of catenated species generally are so great that it appears necessary to abandon the hypothesis. On the other hand, on the hypothesis that the chromosomes occupy fixed positions in the ring, chromosome catenation will explain the general phenomena of genetic linkage so characteristic of Oenothera hybrids.

In the earlier papers on catenation the configuration was regarded as practically constant for each species, hybrid or mutation; but almost from the first occasional exceptions were recorded, and in *O. Agari,* a member of the subgenus Raimannia, much variation in configura-

tion was observed (Sheffield, 1927) although the species breeds strikingly true. Recent papers (*e.g.*, Illick, 1929) show a certain amount of variation, two or three configurations appearing in the pollen mother-cells of a particular phenotype. The most remarkable range of catenation appears, however, in *O. rubricalyx × eriensis*, recently worked out in my laboratory (Hedayetullah, 1932). Most of the hybrids from this cross die in the seedling stage, but the few survivors show a catenation which is frequently a ring of ten and a ring of four. More numerous pollen mother-cells showed a chain of 8+ chain of 4+ chain of 2. But in any of these groups a ring could replace a chain or *vice versa*. A single cell contained a ring of 14 and several a chain of 14, and other arrangements were observed as well.

This brings us to the interpretation of the cytological nature of chromosome linkage. It is known to arise through crossing in Oenothera, and it is reasonable to assume that in Datura and Pisum and probably in Oenothera it has arisen through segmental interchange. A favorite hypothesis in recent papers has been that it always results from segmental interchange between non-homologous chromosomes, with terminalization of the resulting chiasmata. This hypothesis has much to be said for it, yet it encounters serious difficulties as a general explanation. The question of telosynapsis or parasynapsis is also clearly involved. For over twenty years Oenothera was regarded as an unshakable case of telosynapsis. Then efforts were made, especially by Darlington (1931b), to develop a hypothesis which would plausibly account for both parasynapsis and catenation by the terminalization of chiasmata. In a recent paper (Gates and Goodwin, 1931) in which a cytological study of meiosis in *O. deserens* and *O. blandina* was made, some evidence in favor of a parasynaptic interpretation was obtained in these two forms which show no catenation. It was also shown that where seven pairs of chromosomes are present there is no continuous spireme preceding diakinesis, but an appearance of such is produced by

the interlocking of ring pairs before they are condensed into the definitive pairs of chromosomes.

The theory of terminalization of chiasmata, however, has always been in a somewhat precarious state so far as Oenothera is concerned, from lack of indubitable observational evidence. The recent results of Hedayetullah (1932) on a ring-forming Oenothera hybrid are practically impossible to interpret on any but a telosynaptic basis. He uses the argument in an early paper of mine (Gates, 1908) that the chromosomes in a ring or chain must represent individuals, and not the lateral joined threads corresponding to two chromosomes and transversely segmented. But he also shows that the double appearance of the chromosomes in diakinesis is due to the presence of two chromonemata. He has demonstrated (Hedayetullah, 1931) in Narcissus root-tips that the chromosomes are double structures throughout the mitotic cycle. Hence it is natural to interpret the two threads visible in Oenothera chromosomes at diakinesis as the two chromonemata of a somatic chromosome. This is also contrary to Darlington's (1931a) hypothesis that meiosis differs from mitosis in that the contraction of the chromosomes in the heterotypic prophase has anticipated the division of each chromosome into two threads. Hedayetullah shows in fact in Narcissus that the chromosomes split not in prophase but in metaphase, the two halves remaining in close juxtaposition until they separate in the succeeding metaphase.

The chromosome connections in Oenothera Hedayetullah regards as due not to terminalization of chiasmata but to portions of the linin thread being laid bare by the aggregation of the chromatin about certain centers in a spireme which is continuous for each ring or chain, thus forming the linked chromosomes. This point of view appears to be supported by the observation of Sheffield (1927) that in *O. eriensis* the connections between the chromosomes in the ring of 14 are usually noticeably longer than in other species. This peculiarity is also found to be inherited in some at least of the hybrids of

O. eriensis (unpublished).   There is no room for an explanation of these facts in the simple idea that the connections arise through the terminalization of chiasmata.

With regard to the relation between catenation and segmental interchange, the following remarks seem pertinent.   That the transposition of a portion of a chromosome can occur in nature seems clearly proved from the genetic comparison of *Drosophila melanogaster* and *D. simulans*.   The evidence shows a similar transposition to have taken place in certain strains of *Datura Stramonium* and *Pisum sativum*.   It is reasonable to suppose that such changes occurred in the early history of Oenothera.   It will be remembered that these early southern forms apparently had large flowers and were therefore much cross-pollinated.   Also in their dispersal northwards new types which had long been separated and had become differentiated by accumulated gene mutations would be brought together again.   If segmental interchange had occurred in the meantime, then catenation would arise in these interspecific hybrids; and in those crosses in which the parents had already acquired small flowers through one or more mutations in this series, the hybrids would go on being inbred.   If they had not acquired balanced lethals they would segregate more homozygous types with less hybrid vigor—types which would generally disappear in competition.   Once balanced lethals had been acquired, the interspecific hybrids would produce only one or two types, which would continue to breed true.[3]   That hybrids between species with complete catenation frequently also show the same condition— a ring of 14—we have recently found from several instances (see Gates and Catcheside, 1932).

In addition to the types of interspecific differentiation already mentioned, it must be remembered that cytoplasmic differences have also appeared, although we

[3] Both Muller (1930) and Haldane (1932) have concluded that an Oenothera-like condition with balanced lethals would only be likely to arise in an inbred organism.   From our point of view it would therefore arise after the mutations for small flowers had appeared.

have no present knowledge as to how they have arisen. It must also be borne in mind that interspecific Oenothera hybrids are usually intermediate or in some sense a blend between the two parent species.

It might be supposed from these facts that there is practically no limit to the number of "new species" which could be formed by bringing into contact unrelated species with complete catenation. That this is not the case is shown, however, by several lines of experimental evidence. Besides the well known phenomenon of blending and breeding true in interspecific Oenothera hybrids, a contrary series of segregation phenomena is coming to light. This consists of (a) chromosome rearrangement so as simultaneously to reduce catenation and heterosis; (b) reversion to the grand-parental species in double reciprocal crosses, as originally shown by de Vries, (c) elimination of certain complex combinations through lethal conditions or, (d) through certation, *i.e.*, competition of the pollen tubes or competition between megaspore complexes. There is evidence that one type of egg is more or less completely excluded in this way in many crosses.

These phenomena enable us to understand why the small-flowered Oenotheras with complete catenation do not form a continuous series, such as one might otherwise expect to arise from occasional crosses occurring in nature.

We may conclude generally that segmental interchange played its part early in the history of the genus, accompanied by intercrossing. Then followed the northward movement. With the appearance of small flowers, came an era of inbreeding accompanied by increasing catenation and therefore perpetuation of the heterozygous conditions, with occasional outcrosses some of which would produce new and constant types. Hybrid vigor has led to ever-increasing heterozygosis of the surviving forms, tending at the same time to increase catenation to a maximum and eliminate the more homozygous derivatives. In the meantime, gene mutations have supplied the neces-

sary material for species differentiation. It seems obvious that hybridization or segmental interchange alone could not do this. There are many points, however, in the relation between gene mutations and segmental interchange which require elucidation, but I shall not consider them here. Can chromosomes become non-homologous through sufficient gene mutations, or is some other process essential? And what is the real basis of chromosome individuality, which retains their unity notwithstanding crossing over and the various forms of translocation? When we know the basis of the unity of the chromosome we may be able to answer these questions.

It is obvious that our study of the phylogeny of the genus Oenothera has only begun. To test the tentative views here put forward we need a very large body of observations, systematic, cytological, genetical and geographical, on the host of highly diversified forms which exist. In order to bring these under experimental study, I suggest that oenotherologists form a concerted scheme for collecting seeds from particular areas so as to obtain an understanding of the Oenotheras which occupy any region. I have been studying and describing forms from different parts of Canada for some years. Another group has been at work in California, others in Michigan, New Jersey and elsewhere. Might not the territory be mapped out in such a way that each group would be responsible for collecting the seeds, through local botanists or otherwise, from each area. In this way the characters and distribution of each type, regarding which we at present know very little in detail, could ultimately be determined. No doubt it would be necessary for each group of workers to specialize in particular local areas over a period of years; but ultimately by such means we should have accumulated a body of genetic, cytological and systematic data which would make possible a survey of the phylogeny of the genus attainable in no other way.

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SOME PECULIAR RELATIONSHIPS BETWEEN ECTOPARASITES AND THEIR HOSTS[1]

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A STUDY of the relationships of parasites to their hosts may be fruitful in a number of important ways. And in particular such a study is of value in judging relationships of the hosts themselves, their geographical distribution, their palaeogeography, and the phylogeny of both hosts and parasites. The reason why a study of the phylogeny of a parasitic group may throw much light upon the phylogeny of the hosts of the group is because parasites (especially fixed parasites) usually evolve with their hosts. Where they do, this type of evolution is termed "paralleled" phylogeny. But parasites do not always remain exclusively with the hosts of a single species, they may spread to other hosts which offer in or upon their bodies a similar "ecological habitat." Because of this ability of parasites occasionally to spread to new hosts their study is of great importance to that of geographical distribution and palaeogeography.

The object of this contribution is not to discuss the details, the scope, or the general results of the host-parasite method of study but to present certain new facts and to summarize and interpret certain old ones that have come to the writer's attention during recent years while studying ectoparasites. It is hoped that this contribution may have its justification in calling to the attention of biologists the opportunities for utilizing the host-parasite method of study, and in stimulating a desire for further cooperation on the part of students of the hosts and those who study their parasites.

HOST PREFERENCES

The sucking lice, or Anoplura, appear to have a high degree of host specificity. Yet recent experiments indi-

cate that when impelled by hunger and the desire for warmth they will accept as host the mammal available.

In testing this point lice, *Pediculus* (*Parapediculus*) *atelophilus* Ewing, of the gray spider monkey, *Ateles geoffroyi,* were taken from their live host at the National Zoological Park, and offered a feeding upon the writer's arm. Far from being repelled by such a foreign host, they readily took to him, and two adult females fed to repletion. This they did in exactly the same manner as does the body louse of man, *Pediculus humanus corporis* Degeer. Following their engorgement they were kept in what appeared to be almost ideal conditions in a breeding cell next to the body, yet they both died in a few hours.

In another experiment lice, *Pedicinus* sp., from a sick baboonlike monkey, *Magus* sp., were transferred to the arm. These also readily took to the human host, and several began pricking the skin. Two of them, an adult female and a nymph, engorged to repletion. When these two were subsequently kept under what appeared to be good living conditions both died in a few hours.

Monkeys and man are primates belonging to the same order of mammals, and having, as is well known, blood and other physiological characteristics of a somewhat similar nature, yet when sucking lice are taken from hosts of other orders and placed on man, results similar to those just described may follow. Thus specimens of the sucking louse of the dog, *Linognathus piliferus* (Burmeister), taken from their canine host were placed on man, and out of 13 individuals used in two trials 3 fed within a short time. One of these, an adult female, fed for 45 minutes and then was removed to a breeding cell. She soon died, the blood appearing to be only partly digested.

These observations on the experimental transfer of sucking lice from their normal to a foreign host are not only of interest as appearing to indicate a lack of acuteness of the senses of smell and taste in these ectoparasites, but they are of significance in showing that sucking lice will accept foreign hosts. In nature they probably

at times make transfers upon contact of foreign with favored host species.

This tendency to accept foreign hosts may explain the presence in America on spider monkeys, species of *Ateles*, of lice that are so similar to the pediculid lice of man that for many years authorities believed that they were identical. All these monkey lice differ in a few minor characters from those found on man. Yet, knowing the tremendous gap in the natural affinities between man and spider monkeys, who would insist that the presence of these pediculid lice on the latter indicates a paralleled phylogeny of hosts and parasites? The only logical conclusion, from the phylogenetic standpoint, is that the spider monkeys obtained their lice for the first time following the arrival in tropical America of the American Indian. That the Indians were hosts of a *Pediculus* is shown by the presence of these lice on prehistoric Indian mummies.

Interpreting Host Phylogeny from a Study of the Relationships of Their Parasites

Professor V. L. Kellogg and the late Professor L. Harrison have been ardent students of the phylogeny of the biting lice and each has pointed out how the evidence obtained from a study of the relationships of the lice may affect, pro and con, the interpretations of the phylogeny of the hosts. Harrison (*2*), in addressing the Linnean Society of New South Wales, in 1928, said:

The ostriches of Africa and the rheas or nandus of South America are commonly supposed by ornithologists to have arisen from quite distinct stocks. But their lice are so similar, and so different from all other bird-lice, that these must have evolved from a common ancestor, and so also must the birds themselves. Evidence derived from lice is confirmed by cestode and nematode parasites of the two groups of birds. Thus a phylogenetic relationship may be established by means of parasites. Equally, a supposed relationship may be refuted. Their lice prove that the penguins are in no way related to any northern group of aquatic birds, but belong in an ancient complex which includes the tinamous, fowls and pigeons; that the kiwis of New Zealand are modified rails, and not struthious birds at all; that the tropic-birds are not steganopodes but terns, and so on.

Without wishing to endorse all these conclusions of Harrison the writer would like to add that the ostriches of Africa and the rheas of South America have the same mite parasite, *Eupterolichus bicaudatus* (Gervais), and that this mite is not known from any other birds.

The Lice of Cowbirds

The differences between related species of biting lice, or Mallophaga, are believed to have arisen largely because of isolation on certain very similar hosts. Such isolation prevents interbreeding of varieties of species or other closely related forms, and allows them to accumulate minute inheritable increments in any direction.

But what are the factors that support this isolation of species and under what conditions are they rendered inoperative? Kellogg (4), as long ago as 1896, emphasized particularly the fact that in the case of non-gregarious wild birds few contacts are made even between individuals of the same host species and are seldom made between individuals of different species. Among individuals of a single species contact is brought about between adults chiefly during mating, and between parents and young during the nesting period, and between young individuals while in the same nest. To what degree this lack of contact operates to restrict a species might be tested by transferring specimens of a louse species from their natural host or hosts to those of other species. This has already been done in a very limited way, yet with the lice of certain birds it is hardly necessary, for in nature we find the experiment being constantly performed for us and under conditions even better than man can devise and on a scale his ambition would hardly lead him to attempt. In nature this experiment is performed by many birds of parasitic habits when they lay their eggs in the nests of other birds. Here the eggs hatch and the young alien finds himself squeezed in the nest amongst nestlings of a different species. He is reared under the optimum conditions for the transfer of para-

sites.   Thus these birds, the cuckoos and the cowbirds and some others, are exposed at the very beginning of life to infestations of many diverse sorts.

But what are the results of this experiment of nature? Have the cowbirds, for example, acquired a diverse assortment of lice?   In order to answer this question a special study has been made of the lice of the common cowbird, *Molothrus ater,* this host having the parasitic habit best developed of any of the cowbirds.   Dr. Herbert Friedmann (*1, p. 189*) in speaking of this species in his book, ''The Cowbirds,'' has stated that it lays its eggs in the nests of no less than 158 species of birds. These belong to 8 orders, 25 families and 103 genera. Many scores of skins of this cowbird were carefully searched by the writer for the presence of lice.   Of this large number only 13 were found to possess any.   Eight of these were infested with a species of *Philopterus* of the type already reported from icterid hosts, being in fact only slightly different, yet specifically distinct, from two species found on our blackbirds.   Of the other five skins, two were infested with a species of *Degeeriella* of a type quite characteristic of icterids, being very similar, yet distinct, from our blackbird-infesting species and even more distinct from our species found on the meadowlark.   One was infested with a species of *Myrsidea,* probably *M. bonariensis* Malcomson, found on a South American cowbird.   A specimen of *Goniocotes,* evidently a straggler, was taken from another skin.   The remaining skin had on it a specimen of *Degeeriella* and a nymph of *Myrsidea.*

Thus it is seen that our common cowbird is only moderately parasitized, and that its lice are those characteristic of the group of birds to which it belongs.[2]   This is certainly a most remarkable condition considering the

[2] Kellogg (3, pp. 478–480, pl. liv, fig. 6) has described from our cowbird a species, (*Docophorus*) *Philopterus transpositus,* which apparently belongs to a group found only on parrots.   I was unable to take this species from the skins examined.   It must be one of rare occurrence on cowbirds.

fact that their young do not have the usual contacts with
their parents during the nesting period, but with the
young, as well as the adults, of many diverse species.
This experiment of nature has given the entomologists
and parasitologists a crucial test of the host-group speci-
ficity of certain types of biting lice. It does not follow,
however, that all other groups have such a tenacious at-
tachment for their original hosts.

The Kangaroo-Dog Louse

It has been stated that parasites may occasionally
leave their natural hosts and transfer to others of a quite
different sort, provided that in so doing they find a simi-
lar "ecological environment." An outstanding example
of this kind is found in the kangaroo-dog louse, *Hetero-
doxus longitarsus* (Piaget). This louse not only is a native
of Australia and an original parasite of the kangaroo,
but all of the members of its subfamily, the Boopinae,
belong exclusively to Australian marsupials. Following
the introduction of kangaroos into this country for zoo-
logical gardens and circuses this louse has spread to dogs
and is now found on these domestic hosts in many parts
of the warmer regions of the world. And where it occurs
on dogs, frequently it is much more abundant than the
original biting louse of the dog, *Trichodectes canis*
Degeer.

The possession, in this instance, by the dog and the
kangaroo of an identical species indicates the contact of
these two diverse types of hosts. But in the case of both
host species the "distribution" which brought about the
contact was effected through the agencies of man. Had
it happened in ancient geological times it would have
implied an overlapping in the natural range of the dog
and of the kangaroo, an implication which would mean
the lack at that time of any natural barrier. Thus the
absence of any large body of water, or of a mountain
chain, or of an extensive desert, may have been indicated
as the conditions should demand.

The Crab Lice

As an example of implied relationship between hosts of closely related parasites there is here cited for the first time the case of the crab louse, genus *Phthirus*. The crab louse of man, *Phthirus pubis* (Linnaeus), which infests the body of its host, favoring particularly the inguinal region, is a most unusual species in which the first five segments of the abdomen are ankylosed, and the abdominal segments are produced laterally into large tubercles. Because of these profound morphological changes it has been placed in a family (Phthiridae) by itself.

The writer a few years ago was most surprised to find a second species of crab louse, which infests the gorilla. This species, *Phthirus gorillae* Ewing, is known only from the egg and nymphal instar. Until adults are taken the full significance of its relationship to the crab louse of man remains in doubt. Yet it is most surprising to learn that these two congeneric species—one from man, the other from the gorilla—should constitute a family distinct and apart from all the other sucking lice. To the writer this is one more fact indicating not only the natural relationship of man to the gorilla but also the great antiquity of both man and the great apes. If the crab lice have evolved upon the great apes and man, as apparently they have done, then this evolutionary period must have been long enough to develop family characters in these louse species.

Our Common Chigger and Its Hosts

For years the writer has been conducting surveys in different sections of the eastern part of the United States so as to establish the host distribution of the common chigger, *Trombicula irritans* (Riley). This is the mite that in its larval stage is so very annoying to man in certain sections of the country during the hot summer months. As a result of this survey, which has included

the examination of many scores of species and hundreds of individuals of land vertebrates, it is believed that a fair picture of the host distribution of this mite has been obtained. Briefly summarized it is as follows:

The common chigger occurs in nature on certain vertebrate groups, in certain or all stages of the life history of the hosts. It parasitizes four of the five classes of vertebrates—Amphibia, Reptilia, Aves, and Mammalia. Yet the most remarkable thing about this distribution is not the occurrence of the parasite upon so many unrelated hosts but its absence from so many of the host species in all of these four classes.

Of the amphibians only young toads were found infested. Of the snakes only certain land species were infested, while all water snakes and all venomous snakes were without the mites. Of the turtles only one species, *Terrapene carolina,* the common box-turtle, was found infested, yet this one turtle host species proved to be a very important source of supply for the chiggers in nature. Of the birds several species were found to harbor chiggers, yet the vast majority of the mites were obtained from a few ground-frequenting land birds such as the Carolina wren and the towhee. Of the mammals the rabbit was found to be the only important host, and nearly all other wild species were without chiggers.

In many instances the absence of the chiggers on certain groups of hosts was easily explained because the habits of the hosts did not expose them to attack. Unattached chiggers occur only in ground litter where there is sufficient depth of the same to insure moisture in the bottom layers for most of the year. Chiggers are probably absent from most amphibians, from water snakes, water turtles, water birds, and other water species because these hosts do not frequent the particular places where the mite larvae are. Birds that feed in the air and build nests above the ground, as well as those that nest and feed exclusively in trees, would not come in contact with chiggers. On the other hand certain game

birds, such as the bob-white, that feed on the ground, nest on the ground, and roost on the ground, are found to be heavily parasitized with chiggers. Young toads are infested with chiggers, but the adults are not. The absence of the mites from the adult toads may be explained by the presence of repellent glands. Similarly the absence of chiggers from many, if not the most, of the mammals may be explained. Chiggers are easily repelled, as has been shown repeatedly, by any oil or other liquid with a moderate to strong odor. Persons who are largely immune to chigger attack frequently may owe this immunity to a body odor.

In casting about to find an analogous case to that of our common chigger in its host distribution a somewhat similar one has been found. It is that of our rabbit-bird tick, *Haemaphysalis leporis-palustris* Packard. This tick, which does not attack man or most of the other mammals, is very abundant on rabbits. Most birds are not attacked by it, but many birds that feed, nest or roost on the ground are parasitized. In addition to not being found on man, the rabbit-bird tick also differs in its host relationships from those of the chigger in not being reported on cold-blooded vertebrates. Thus, up to date the host distribution of our common chigger appears to be the most unusual in its "spotted" diversity of that of any ectoparasitic species yet studied.

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SHORTER ARTICLES AND DISCUSSION

RELATION OF CHROMATID CROSSING OVER TO THE UPPER LIMIT OF RECOMBINATION PERCENTAGES[1]

THERE is abundant evidence from Drosophila and other organisms that as the map distance between genes increases the recombination percentage increases toward 50 as an upper limit. In linkage group I of maize, for example, the map distances and recombination values for certain genes are approximately as follows:

Map distances		Recombination values	
P	0		
as	25	$P-as$	25
f_1	58	$P-f_1$	41
an	75	$P-an$	45
bm_2	127	$P-bm_2$	49

The differences between map distances and the percentages of recombination have been accounted for by the occurrence of multiple crossovers (Morgan, 1919). Jennings (1923) has shown that crossing over without interference or with interference extending on the average over a distance of not more than 30 map units should give recombination values not exceeding 50 per cent. These conclusions were arrived at on the basis of the then accepted hypothesis that crossing over takes place between undivided chromosomes. It is important, therefore, to point out the obvious, but to the best of our information unpublished, relation between the occurrence of chromatid crossing over and the fact that the percentage of recombination between two linked genes, irrespective of how great their map distance may be, does not in general exceed 50. It is our contention that random chromatid crossing over, rather than multiple cross-overs with restricted interference, is the fundamental mechanism which results in recombination percentages approaching 50 as a limiting value.

[1] Paper No. 198, Department of Plant Breeding, Cornell University, Ithaca, N. Y.

374

It has been shown in Drosophila, Zea and Habrobracon that crossing over occurs when the chromosomes are split equationally into two strands or chromatids. It has further been shown by the work of Anderson (1925) and Bridges and Anderson (1925) that only two strands exchange parts, or cross over, at any one level. Therefore at each point of crossing over there are two crossover and two non-crossover strands. If in a given bivalent there is always one point of crossing over between two loci it follows that 50 per cent. of the strands will be crossover strands and 50 per cent. will be non-crossover strands and the percentage of recombination between the two loci will be 50. If two points of crossing over occur between the two loci and if, as the Drosophila data on attached-X's suggest, the identity of the two strands involved in the second point of crossing over is not influenced by the identity of the two strands involved in the first point of crossing over, the proportion of resulting strands is 1 non-crossover : 2 single crossovers : 1 double crossover. Since the double crossover strands have the two loci in the parental combination the ratio of parental combination strands to recombination strands is 1:1. The following table, which was taken from Belling (1931), shows the relation between the number of crossover points to the frequency with which different types of strands are recovered.

Number of crossover points	Types of recovered strands					
	0	1	2	3	4	5
	Non-cross-over-strands	Single crossover strands	Double crossover strands	Triple crossover strands	Quadruple crossover strands	Quintuple crossover strands
1	16	16				
2	8	16	8			
3	4	12	12	4		
4	2	8	12	8	2	
5	1	5	10	10	5	1

As the above table shows, the ratio of parental combination to recombination strands is always 1:1, irrespective of the number of crossover points which occur between the loci of any two genes whose inheritance is being studied. Therefore, the

percentage of recombination between any two genes, which is determined by the ratio of recombination strands to the total number of strands, can never exceed 50. Since Belling's table is merely the expansion of $(a+b)^n$, where "a" stands for non-recombination strands, "b" for recombination strands, and "n" for number of crossover points, it should give the same result as chromosome crossing over without interference, as calculated by Jennings (1923) from the same formula. The important consideration here is that these calculations for results of chromatid crossing over are quite independent of the relative frequency of single, double and other multiple crossovers, *i.e.*, independent of interference. They are true for no interference, for complete interference, and for any degree of interference. Furthermore, they hold whether or not crossing over occurs between sister strands.

These conclusions are true only if crossing over between the different strands at the different crossover points is random,[2] as the Drosophila data on attached X's indicate. If, however, crossing over is not random; if, for example, in a bivalent chromosome in which two crossover points have occurred, chromatids a′ and b were involved in the first crossover point and chromatids a and b′ were always involved in the second crossover point, there would result four single-crossover strands. With such a selective mechanism it would be possible to have more than 50 per cent. of recombinations. This might, perhaps, explain the data of Clausen (1926) on Viola and of Wellensiek (1929) on Pisum where more than 50 per cent. of recombination was found.

<div align="right">

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[2] Crossing over can be made non-random by the exclusion of sister-strand crossovers without influencing the results.

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FURTHER EVIDENCE OF LINKAGE IN SIZE INHERITANCE

In a former communication to this journal, the writer (1931) was able to show an indubitable association in heredity between certain size and color characters in the back-cross generation of a mouse interspecific cross (*Mus musculus* x *Mus bactrianus*). The greater weight, humerus, femur and tibia lengths and body length, characteristic of the recessive *musculus* parent, were found associated with brown coat color derived from the same parent, while greater body and tail lengths likewise tended to be characteristic of those animals manifesting the recessive gene for dilution. In those instances in which the mean difference between dominants and recessives equaled or exceeded four times its probable error, genetic linkage between quantitative and qualitative characters was considered as demonstrated. Since such linkage had not previously been demonstrated in mammals, this interpretation did not meet the unqualified approval of all geneticists (Castle, 1932). Hence, further evidence derived from a study of adult weight in the F_2 generation of the same cross may be of interest.

The use of weight as a measure of the size of an organism as a whole, while not entirely satisfactory because of fluctuations brought about by the amount of food ingested, degree of obesity, etc., has the advantage of being easier to obtain than other criteria of size. In this note, weights of *bactrianus, musculus* and F_1 (*musculus* ♀ × *bactrianus* ♂), backcross and F_2 hybrids

are considered.   All back-cross mice and nearly all animals of
the other generations were weighed on the 181st day of life.
The exceptions were weighed from one to three days later.   All
individuals included were born and raised in our laboratory at
Bar Harbor, Maine.

Since a great majority of the F_1 and F_2 animals were females
(having been produced primarily for a study of spontaneous
tumors arising in old age), the weights of all male mice used in
this report have been converted into female equivalents and
both sexes treated together.   These were obtained by dividing
the mean female weight of each generation by the mean male
weight and then multiplying the weights of the individual males
by the quotient obtained.   These quotients varied from .829 for
bactrianus to .899 for the back-cross mice.   Previous studies
have revealed no sex-linked factors influencing weight, so the
procedure is probably justifiable.

123 individuals of *Mus bactrianus* possessed a mean weight of
$13.0 \pm .11$ grams with a range extending from 9.6 to 18.3 grams
while 116 *musculus* had a mean of $22.4 \pm .19$ grams, ranging
from 15.2 to 30.8.   There is consequently a slight degree of over-
lapping in the two forms.   70 F_1 mice were intermediate in
weight, although nearer to the larger parent, with a mean of
$20.2 \pm .21$ grams and a range from approximately 13 to 27
grams.   The back-cross generation, consisting of 293 individuals,
varied in weight from 13 to 31 grams with a mean of $19.5 \pm .12$.
219 F_2's ranged from 7 to 35 grams with a mean of $18.9 \pm .21$,
thus extending beyond the limits of the two parent species.

The coefficients of variation show the following results:

Generation	Coefficient of variation
bactrianus	$13.78 \pm .60\%$
musculus	$13.82 \pm .62\%$
F_1	$12.67 \pm .73\%$
back-cross	$15.58 \pm .44\%$
F_2	$23.82 \pm .81\%$

From these figures it appears that the parent races and the F_1
hybrids differ but little in variability.   The back-cross mice, on
the contrary, are more variable than the F_1, possibly signifi-
cantly so since the difference is 3.4 times its probable error.   The

F_2 generation is much more variable, the difference being 10.2 times its probable error.

Since the parent species differ in three pairs of color genes, the back-cross of the F_1 to the triple recessive *musculus* produces approximately equal numbers of dominant and recessive members of each of the factor pairs. If the "marked" *musculus* chromosomes carrying the recessive genes for color also possess genes influencing weight, the back-cross mice showing the recessive characters will tend to be heavier than those with the dominant allelomorphs. If size is not influenced through chromosomal genes, or if no such genes are present on any of the three "marked" chromosomes, the recessive members of the factor pairs will exhibit no tendency to exceed the dominant. All intense mice were compared with all dilute, black with brown, and agouti with non-agouti with the results listed below.

No.	Color	Mean Weight	Difference	Difference ——————— Probable error
149	Intense	19.5 ± .17 grams		
144	Dilute	19.5 ± .17 "	0.0 ± .24 grams	0.0
143	Black	18.6 ± .15 "		
150	Brown	20.3 ± .17 "	1.7 ± .23 "	7.4
155	Agouti	19.3 ± .15 "		
138	Non-agouti	19.7 ± .19 "	0.4 ± .24 "	1.7

In the F_2 generation, three times as many dominants as recessives are expected in each of the factor pairs. A comparison of the two classes gives the following figures:

No.	Color	Mean Weight	Difference	Difference ——————— Probable error
168	Intense	19.0 ± .25 grams		
51	Dilute	18.8 ± .33 "	−.2 ± .41 grams	0.5
166	Black	18.3 ± .21 "		
53	Brown	20.7 ± .49 "	2.4 ± .53 "	4.5
165	Agouti	18.5 ± .22 "		
54	Non-agouti	20.2 ± .47 "	1.7 ± .52 "	3.3

From the back-cross and F_2 data it appears obvious that there is no difference in the weights of intense and dilute mice. The browns, however, are heavier than the blacks to an undoubtedly significant degree. In the comparison of agoutis and non-agoutis the situation is less clear. Non-agouti mice are heavier than agouti in both generations but only slightly so in the back-cross, while the difference in the F_2 generation is merely of doubtful significance. The data, however, are confirmatory of the former finding, at least in so far as adult weight is concerned, that genes influencing size are found on the chromosome with the gene for brown coat color.

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A COMPARATIVE ANATOMICAL STUDY OF A MUTANT AQUILEGIA

An anatomical study of the *"compacta"* mutants of the European columbine, *Aquilegia vulgaris,* has yielded indirect evidence as to the possible action of the responsible gene. A more complete analysis is under way; in the meantime the pertinent facts are summarized below.

Description

The homozygous recessive *compactas* differ from normal garden columbines (*A. vulgaris*) in a number of ways. (1) They are shorter, bushier and much more branched. In Fig. 1 are shown diagrammatic drawings, made to scale from actual specimens. (2) The stems are more brittle than those of the normal plant, so much so that in walking about in the experimental garden one has to be careful not to brush them roughly, a precaution quite unnecessary with other Aquilegias. (3) The most striking peculiarity of *compacta* is the position of the flower. In normal *A. vulgaris* the bud droops and not until the petals

are about to fall does the flower become erect.   In the *compacta*
mutants the bud is erect even before the first anthers have
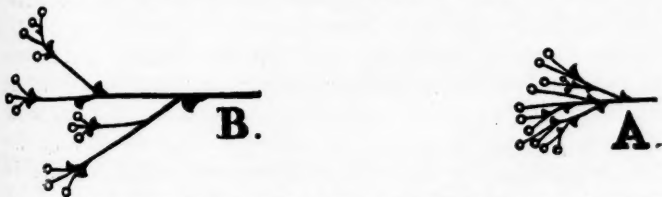opened.   (4) In families segregating for *compacta* and non-

Fig. 1. Diagrammatic drawings (to scale) of the main shoots of normal
(B) and mutant (A) Aquilegias. Circles represent flowers
and triangles represent leaves.

compacta it is possible to illustrate a fourth difference, a slight
effect on the proportions of the petals and the sepals.   The
laminae of the petals are shorter in the mutants and the sepals,
on the average, are less well developed.

ORIGIN

Nothing is known as to the ultimate origin of the mutation
other than the fact that it is commonly found among cultivated
Aquilegias.   A dwarf white variety, homozygous for *compacta*,
is offered by a number of seed houses under the name of "*alba
nana compacta*."   In its general features the mutant resembles
the "*compacta*" or "*nana compacta*" freaks, known for a large
number of cultivated plants.

INHERITANCE

Compacta behaves as a simple Mendelian, single-factor reces-
sive.   The material with which we are working was originally
given us by Miss Brenhilda Schafer, of the John Innes Horticul-
tural Institution (Anderson and Schafer, 1931), who has gra-
ciously permitted us to refer to her unpublished results.

ANATOMY

An anatomical study was undertaken to determine the struc-
tural basis for the difference in height and rigidity between the
normals and the *compactas*. Sections of the stem were cut from
the following parts of the plants: (1) The base of the central
shoot (cross and longitudinal sections) ; (2) the peduncle of the

bud, taken at a point 2 cm. below the base of the bud (cross-sections) ; (3) the peduncle of the fruit, taken at a point 2 cm. below the base of the fruit (cross-sections).

For both normal and dwarf plants two genetic types were used; one having blue flowers and one with white flowers.

The anatomical structure of the mature Aquilegia stem as seen in cross-section is briefly as follows: A large soft pith is surrounded by a ring of bicollateral vascular bundles, each with a cap of schlerenchyma over its outer surface. Encircling the ring of bundles is a sheath of schlerenchyma, which in turn is clothed with a narrow layer of thin-walled parenchyma and an epidermis.
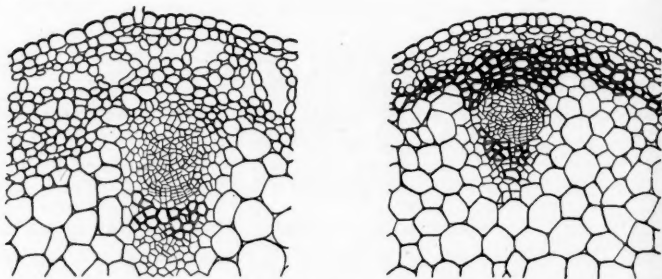

Fig. 2.   Cross-sections of the peduncles of young buds of normal (left) and mutant (right) Aquilegias.

The most noticeable difference between cross-sections of normal and dwarf stems was obvious under a hand lens.   In the base of the stem from the normal plant, the thick-walled, small-lumened cells capping the bundles are often separated from those of the outer layer by intervening large-lumened cells, while in the same part of the dwarf plant the bundle caps are continuous with the outer schlerenchymatous sheath.   The secondary walls of the cortex schlerenchyma are thicker in the dwarf than in the normal type.   The metaxylem elements appear to have slightly thicker secondary walls in the dwarf plants.

The contrast between normal and dwarf plants is greater in the younger stems (Fig. 2) than in the mature tissues (Fig. 3). Stiffening of the stem seems to occur at an earlier stage in the *compactas* than in the normals.   Cross-sections of the peduncle at equal distances below the buds of normal and *compacta* plants showed the basis for this difference.   At a distance of 2 cm from

the bud the peduncle of the normal plant showed no secondary thickening of the cell wall in the cortex. Secondary thickening of the schlerenchyma in the same part of the peduncle of the dwarf plant was marked.

The peduncle of the fruit is rigid in both dwarf and normal plants, although the peduncle in the dwarf type is the stiffer of the two. Anatomical structure bears this out. Both peduncles show a cap of thick-walled, small-lumened cells over the vascular
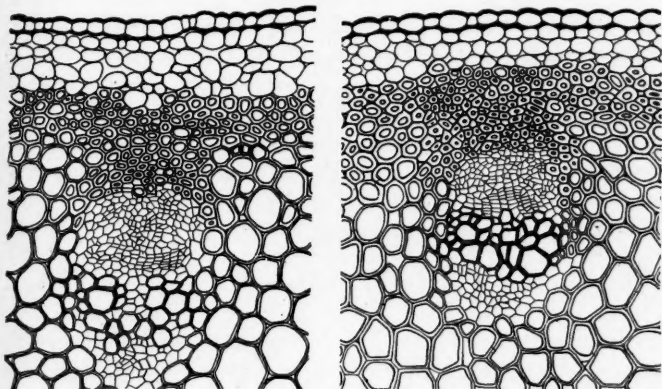

FIG. 3. Cross-sections of the main shoots of normal (left) and mutant (right) Aquilegias.

bundle, but in the normal type the schlerenchymatous ring in the cortex is made up of moderately thick-walled but large-lumened cells, while in the dwarf type the ring is made up of cells similar in cross-section to those of the cap.

CONCLUSION

The evidence so far obtained suggests that the manifold effects of the *compacta* gene are all directly or indirectly the result of precocious secondary thickening of the cell walls. Since thickening begins earlier in the *compactas* the flower is upright at a much earlier stage. Since it begins before elongation is complete, it results directly in dwarfism and indirectly in a bushy, compact habit of growth. When the main stem can elongate no further the side branches are pushed out and these in turn stiffen precociously, giving rise to vigorous tertiary branches. Side-growth continues in this way, resulting in a plant form

similar in origin and in appearance to a hedge plant which has been repeatedly sheared by the gardener. The greater brittleness of stem in the mutant is probably another result of the greater secondary thickening which, beginning at an earlier stage, reaches a more extreme condition.

An increasing number of Mendelian mutants have been shown to support Goldschmidt's (1927) generalization that genes affect times and rates of development. Particularly interesting by way of contrast to our case is the study by Jenkins and Gerhardt (1931) of the "lazy" gene in maize. There the mutant plants differ from the normals in that secondary thickening of the cell wall takes place at an abnormally *late* stage in stem development.

The anatomical evidence presented above suggests that the *compacta* mutants differ from the normals only in the fact that secondary thickening of the cell walls begins at an earlier stage in stem development. Secondary thickening is *apparently* continued as long in the mutants as in the normals. The secondary wall is *apparently* of the same constitution in the mutants as in the normals. Secondary thickening begins so much earlier in the mutants that the difference is *apparently* one of time of development rather than rate of development. Critical evidence on these three points is still lacking. Provisionally, we may conclude that the *compacta* gene causes its peculiar effects through the precocious initiation of a normal feature of normal development.

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